XJENZA ONLINE
Special Issue, 2017

Mediterranean Neuroscience Society
www.mnsociety.net

MALTA NEUROSCIENCE NETWORK

MNS MALTA 2017

6th Mediterranean Neuroscience Society Conference 2017

MALTA MNS2017

Radisson BLU, St Julian's, Malta, June 12 – 15, 2017

Organised by the Mediterranean Neuroscience Society (MNS) Hosted by Malta Neuroscience Network, University of Malta, Malta

http://www.mnsmeeting2017.com/

The University of Malta Research, Innovation & Development Trust

Conference Proceedings

Malta Chamber of Scientists

www.xjenza.com

The Journal of the Malta Chamber of Scientists

Editor-in-Chief: Giu[illegible]e Di Giovanni

Mediterranean Neuroscience Society

Why a MNS?

Research on brain function in health and disease is among the priorities for today's societies, and several indicators put the Mediterranean research area among strategic issues for building new profitable relationships. Many South-North collaborations and networks have emerged in recent years through bilateral and multi-lateral actions, supported by the EU or by international and national actions, whether for setting up teaching curricula, or by building human potential. The MNS is created to support and help strengthening all initiatives that bring together Mediterranean neuroscientists.

The last MNS conference was organized in Sardinia (Italy), after four previous successful editions (Montpellier 1997; Marrakech 2006, Alexandria 2009 and Istanbul 2012). These conferences gather up to 400 scientists from all Mediterranean and other countries, and offer a rich program with world-class lectures, symposia, poster sessions and social events. These meetings have proved to be highly beneficial, not only for the scientific exchanges, but also in terms of training opportunities for students and young researchers.

Objectives of the MNS

The MNS works towards three main objectives:

- Strengthen exchanges between Mediterranean neuroscientists
- Promote education in Neuroscience and increase public awareness
- Sustain the Mediterranean Neuroscience Conference

To reach these objectives, the MNS's policy is to work in close cooperation with existing national and international Neuroscience and Scientific Societies.

Contact information

Mediterranean Neuroscience Society – MNS

http://www.mnsociety.net/

Dr. Youssef Anouar, Secretariat of the MNS - INSERM U982, DC2N, Faculté des Sciences, Université de Rouen, 76821 Mont-Saint-Aignan Cedex, France.

E-mail: youssef.anouar@univ-rouen.fr

Xjenza Online - Journal of Malta Chamber of Scientists
http://www.xjenza.org

Editorial Board

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Chronological List of Past and Present Editors of Xjenza The Journal of the Malta Chamber of Scientists

2013–
Editor: Giuseppe Di Giovanni
Associate Editors: *David Magri, Ian Thornton, Ian Cassar, Philip Farrugia, Sebastiano D'Amico, Nicholas Sammut, David Mifsud, Godfrey Baldacchino, Liberato Camilleri, Carmel Cefai*
Xjenza Online Vol. 4 Iss. 2 - December 2016
Xjenza Online Vol. 4 Iss. 1 - July 2016
Xjenza Online Vol. 3 Iss. 2 - December 2015
Associate Editors: *David Magri, Ian Thornton, Ian Cassar, Philip Farrugia, Sebastiano D'Amico, Nicholas Sammut, Joseph Galea, David Mifsud, Sandro Lanfranco, Mario Valentino, Godfrey Baldacchino, Liberato Camilleri*
Xjenza Online Vol. 3 Iss. 1 - August 2015
Xjenza Online Vol. 2 Iss. 2 - October 2014
Xjenza Online Vol. 2 Iss. 1 - March 2014
Xjenza Online Vol. 1 Iss. 2 - October 2013
Xjenza Online Vol. 1 Iss. 1 - March 2013

2003–2007
Editors: Joseph N. Grima and Richard Muscat
Xjenza Vol. 12 - 2007
Xjenza Vol. 11 - 2006
Xjenza Vol. 10 - 2005
Xjenza Vol. 9 - 2004
Xjenza Vol. 8 - 2003

1996–2002
Editor: Angela Xuereb
Associate Editor: *Richard Muscat*
Xjenza Vol. 7 - 2002
Xjenza Vol. 6 - 2001
Associate Editors: *Martin Ebejer and Richard Muscat*
Xjenza Vol. 5 - 2000
Xjenza Vol. 4 Iss. 2 - 1999
Xjenza Vol. 4 Iss. 1 - 1999
Associate Editors: *Martin Ebejer, Richard Muscat, and Christian A. Scerri*
Xjenza Vol. 3 Iss. 2 - 1998
Xjenza Vol. 3 Iss. 1 - 1998
Associate Editors: *Martin Ebejer, Richard Muscat, Christian A. Scerri and Emmanuel Sinagra*
Xjenza Vol. 2 Iss. 2 - 1997
Xjenza Vol. 2 Iss. 1 - 1997
Xjenza Vol. 1 Iss. 2 - 1996
Xjenza Vol. 1 Iss. 1 - 1996

Scope of Journal

Xjenza is the Journal of the Malta Chamber of Scientists and is published in an electronic format. Xjenza is a peer-reviewed, open access international journal. The scope of the journal encompasses research articles, original research reports, reviews, short communications and scientific commentaries in the fields of: mathematics, statistics, geology, engineering, computer science, social sciences, natural and earth sciences, technological sciences, linguistics, industrial, nanotechnology, biology, chemistry, physics, zoology, medical studies, electronics and all other applied and theoretical aspect of science.

The first issue of the journal was published in 1996 and the last (No. 12) in 2007. The new editorial board has been formed with internationally recognised scientists, we are planning to restart publication of Xjenza, with two issues being produced every year. One of the aims of Xjenza, besides highlighting the exciting research being performed nationally and internationally by Maltese scholars, is to provide insight to a wide scope of potential authors, including students and young researchers, into scientific publishing in a peer-reviewed environment.

Instructions for Authors

Xjenza is the journal of the Malta Chamber of Scientists and is published by the Chamber in electronic format on the website: http://www.mcs.org.mt/index.php/xjenza. Xjenza will consider manuscripts for publication on a wide variety of scientific topics in the following categories

1. Communications
2. Research Articles
3. Research Reports
4. Reviews
5. Notes
6. News and Views
7. Autobiography

Communications are short peer-reviewed research articles (limited to three journal pages) that describe new important results meriting urgent publication. These are often followed by a full Research Article.

Research Articles form the main category of scientific papers submitted to Xjenza. The same standards of scientific content and quality that applies to Communications also apply to Research Articles.

Research Reports are extended reports describing research of interest to a wide scientific audience characteristic of Xjenza. Please contact the editor to discuss the suitability of topics for Research Reports.

Review Articles describe work of interest to the wide readership characteristic of Xjenza. They should provide an in-depth understanding of significant topics in the sciences and a critical discussion of the existing state of knowledge on a topic based on primary literature. Review Articles should not normally exceed 6000 words.Authors are strongly advised to contact the Editorial Board before writing a Review.

Notes are fully referenced, peer-reviewed short articles limited to three journal pages that describe new theories, concepts and developments made by the authors in any branch of science and technology. Notes need not contain results from experimental or simulation work.

News and Views: The News section provides a space for articles up to three journal pages in length describing leading developments in any field of science and technology or for reporting items such as conference reports. The Editor reserves the right to modify or reject articles for consideration as 'news items'.

Commentaries: Upon Editor's invitation, commentaries discuss a paper published in a specific issue and should set the problems addressed by the paper in the wider context of the field. Proposals for Commentaries may be submitted; however, in this case authors should only send an outline of the proposed paper for initial consideration. The contents of the commentaries should follow the following set of rules: 3000 words maximum, title 20 words maximum, references 10 maximum (including the article discussed) and figures/tables 2 maximum.

Errata: Xjenza also publishes errata, in which authors correct significant errors of substance in their published manuscripts. The title should read: Erratum: "Original title" by ***, Xjenza, vol. *** (year). Errata should be short and consistent for clarity.

Invited Articles and Special Issues: Xjenza regularly publishes Invited Articles and Special Issues that consist of articles written on invitation by the Editor or member of the editorial board.

Submission of Manuscripts

Manuscripts should be sent according to the guidelines given hereafter to submissionxjenzaonline@gmail.com.

Referees All manuscripts submitted to Xjenza are peer reviewed. Authors are requested to submit with their manuscript the names and addresses of three referees, preferably from overseas. Every effort will be made to use the recommended reviewers; however the editor reserves the right to also consult other competent reviewers.

Conflict of Interest Authors are expected to disclose any commercial or other associations that could pose a conflict of interest in connection with the submitted manuscript. All funding sources supporting the work, and institutional or corporate affiliations of the authors, should be acknowledged on the title page or at the end of the article.

Policy and Ethics The work described in the submitted manuscript must have been carried out in accordance with The Code of Ethics of the World Medical Association (Declaration of Helsinki) for experiments involving humans (`http://www.wma.net/en/30publications/10policies/b3/index.html`); EU Directive 2010/63/EU for animal experiments (`http://ec.europa.eu/environment/chemicals/lab_animals/legislation_en.htm`); Uniform Requirements for manuscripts submitted to Biomedical journals (`http://www.icmje.org`). This must be stated at an appropriate point in the article.

Submission, Declaration and Verification Submission of a manuscript implies that the work described has not been published previously (except in the form of an abstract or as part of a published lecture or academic thesis), that it is not under consideration for publication elsewhere, that it has been approved for publication by all authors, and tacitly or explicitly, by the responsible authorities where the work was carried out, and that, if accepted, it will not be published elsewhere in the same form, in English or in any other language, including electronically, without the written consent of the copyright-holder.

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Preparation of Manuscripts

Xjenza accepts submissions in MS Word, Libre Office Writer and LaTeX with the latter being the preferred option. Anyone submitting in LaTeX should use the journal template, the latest version of which can be found at `http://github.com/hicklin/Xjenza-Journal-Template`. All the necessary files to run the LaTeX document should be supplied together with the rendered PDF.

If a word processor is used the styling should be kept to a minimum, only introducing bold face, italics, subscript and superscript text where the context requires it. Text should be in single-column format and the word processor options should not be used in order to justify text or hyphenate words. Together with the native format of the word processor, a pdf, generated by the word processor, must be given. Furthermore, artwork should be in accordance to the artwork guidelines give below and must be submitted separately from the word processor file. Similarly, the bibliographic data of the cited material should be submitted separately as an Endnote (*.xml), Research Information Systems (*.ris), Zotero Library (zotero.splite) or a BiBTeX (*.bib) file.

Article Structure

A manuscript for publication in Xjenza will ordinarily consist of the following: Title page with contact information, Abstract, Highlights, Keywords, Abbreviations, Introduction, Materials and Methods, Results, Discussion, Conclusion, Appendices and References.

The manuscript will be divided into clearly defined numbered sections. Each numbered subsection should be given a brief heading. Each heading should appear on its own separate line. Subsections should be used as much as possible when cross-referencing text: refer to the subsection by the section number as opposed to simply 'the text'.

Title page

- Title should be concise yet informative. Titles are often used in information-retrieval systems. Avoid abbreviations and formulae where possible.
- Author names and affiliations. Present the authors' affiliation addresses (where the actual work was done) below the names. Indicate all affiliations with a lower-case superscript number immediately after each author's name and in front of the appropriate address. Provide full postal address of each affiliation, including the country name and, if available, the e-mail address.
- Corresponding author. Clearly indicate who will handle correspondence at all stages of refereeing and publication, including post-publication. Ensure that telephone and fax numbers (with country and area code) are provided in addition to the e-mail address and complete postal address. Contact details must be kept up to date by the corresponding author.
- Present/permanent address. If an author has changed the address since the work described, this can be indicated as a footnote to the author's name. The address at which the author actually did the work must be retained as the main, affiliation address. Superscript Arabic numerals are used for such footnotes.

Abstract A concise and factual abstract is required of up to about 250 words. The abstract should state briefly the background and purpose of the research, the principal results and major conclusions. An abstract is often presented separately from the article, so it must be able to stand alone. For this reason, references and non-standard abbreviations should be avoided. If essential, these must be defined at first mention in the abstract itself.

Abbreviations Define abbreviations that are not standard in this field in a footnote to be placed on the first page of the article. Such abbreviations that are unavoidable in the abstract must be defined at their first mention as well as in the footnote and should be used consistenly throughout the text.

Introduction State the objectives of the work and provide an adequate background, avoid a detailed literature survey or a summary of the results.

Materials and Methods Provide sufficient detail to allow the work to be reproduced. Methods already published should be indicated by a reference: only relevant modifications should be described.

Results Results should be clear and concise. Numbered/tabulated information and/or figures should also be included.

Discussion This should explore the significance of the results of the work, yet not repeat them. Avoid extensive citations and discussion of published literature. A combined section of Results and Discussion is often appropriate.

Conclusion The main conclusions based on results of the study may be presented in a short Conclusions section. This may stand alone or form a subsection of a Discussion or Results and Discussion section.

Appendices Formulae and equations in appendices should be given separate numbering: Eq. (A.1), Eq. (A.2), etc.; in a subsequent appendix, Eq. (B.1) and so on. Similarly for tables and figures: Table A.1; Fig. A.1, etc.

Acknowledgements Collate acknowledgements in a separate section at the end of the article before the references and do not, therefore, include them on the title page, as a footnote to the title or otherwise. List here those individuals who provided assistance during the research (e.g., providing language help, writing assistance or proof reading the article, etc.).

Units Follow internationally accepted rules and conventions: use the international system of units (SI). If other units are mentioned, please give their equivalent in SI. Anyone using LaTeX should use the package siunitx in all cases.

Footnotes Footnotes should be used sparingly. Number them consecutively throughout the article, using superscript Arabic numbers. Many word processors build footnotes into the text, and this feature may be used. Should this not be the case, indicate the position of footnotes by a superscript number in the text and present the footnotes themselves separately at the end of the article. Do not include footnotes in the Reference list.

Table Footnotes Indicate each footnote in a table with a superscript lower case letter.

Artwork Electronic artwork General points:

- Make sure you use uniform lettering and sizing of your original artwork.
- Save text in illustrations as 'graphics' or enclose the font.
- Only use the following fonts in your illustrations: Arial, Courier, Times, Symbol or Computer Modern Roman, the latter is preferred.
- Number the illustrations according to their sequence in the text.
- Name your artwork files as 'fig$x$' or 'tab$x$' where x corresponds to the sequence number in your document.
- Provide captions to illustrations separately.
- Produce images near to the desired size of the printed version or grater.
- Make sure that the artwork has no margins and borders.
- Submit each figure as a separate file.

Formats Regardless of the application used, when your electronic artwork is finalised its file format should be one of the following (note the resolution requirements for line drawings, halftones, and line/halftone combinations given below):

- PDF or SVG: Vector drawings. Embed the font or save the text as 'graphics'.
- JPEG or PNG: Color or grayscale photographs (halftones): always use a minimum of 300 dpi.
- JPEG or PNG: Bitmapped line drawings: use a minimum of 1000 dpi.
- JPEG or PNG: Combinations bitmapped line/half-tone (color or grayscale): a minimum of 500 dpi is required.

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Tables Number tables consecutively in accordance with their appearance in the text. Place footnotes to tables below the table body and indicate them with superscript lowercase letters. Avoid vertical rules. Be sparing in the use of tables and ensure that the data presented in tables do not duplicate results described elsewhere in the article. Large tables should be submitted in CSV format.

Citations and References Reference and citation styles for manuscripts submitted to Xjenza should be in accordance to the APA v6 style.

Citation in text References to cited literature in the text should be given in the form of an author's surname and the year of publication of the paper with the addition of a letter for references to several publications of the author in the same year. For further information regarding multiple authors consult the APA v6 guidelines. Citations may be made directly

Kramer et al. (2010) have recently shown ...

or parenthetically

as demonstrated (Allan, 2000a, 2000b, 1999; Allan and Jones, 1999).

Groups of references should be listed first alphabetically, then chronologically. When writing in LaTeX use `\textcite{}` and `\parencite{}` for the respective cases mentioned.

The reference section Every reference cited in the text should also be present in the reference list (and vice versa). The reference list should also be supplied as an Endnote (*.xml), Research Information Systems (*.ris), Zotero Library (zotero.splite) or a BiBTeX (*.bib) file. Unpublished results and personal communications are not recommended in the reference list, but may be mentioned in the text. If these references are included in the reference list they should follow the standard reference style of the journal and should include a substitution of the publication date with either 'Unpublished results' or 'Personal communication'. Citation of a reference as 'in press' implies that the item has been accepted for publication.

References should be arranged first alphabetically and then further sorted chronologically if necessary. More than one reference from the same author(s) in the same year must be identified by the letters 'a', 'b', 'c', etc., placed after the year of publication. Consult the APA v6 guidelines for multiple authors. Below are some examples of referencing different bibliographic material.

Reference to a Journal Publication:

Agree, E. M. and Freedman, V. A. (2011). A Quality-of-Life Scale for Assistive Technology: Results of a Pilot Study of Aging and Technology. *Phys. Ther.*, 91(12):1780–1788.

McCreadie, C. and Tinker, A. (2005). The acceptability of assistive technology to older people. *Ageing Soc.*, 25(1):91–110.

Reference to a Book:

Brownsell, B. (2003). *Assistive Technology and Telecare: Forging Solutions for Independent Living.* Policy Press, Bristol.

Fisk, M. J. (2003). *Social Alarms to Telecare: Older People's Services in Transition.* Policy Press, Bristol, 1st edition.

Reference to a Chapter in an Edited Book:

Brownsell, S. and Bradley, D. (2003). New Generations of Telecare Equipment. In *Assist. Technol. Telecare Forg. Solut. Indep. Living*, pages 39–50.

Web references The full URL should be given together with the date the reference was last accessed. Any further information, if known (DOI, author names, dates, reference to a source publication, etc.), should also be given. Web references can be listed separately or can be included in the reference list.

References in a Special Issue Please ensure that the words 'this issue' are added to any references in the list (and any citations in the text) to other articles in the same Special Issue.

Journal Abbreviations Journal names should be abbreviated according to:

-Index Medicus journal abbreviations: `http://www.nlm.nih.gov/tsd/serials/lji.html`;

-List of title word abbreviations: `http://www.issn.org/2-22661-LTWA-online.php`;

-CAS (Chemical Abstracts Service): `http://www.cas.org/sent.html`.

Video data Xjenza accepts video material and animation sequences to support and enhance the presentation of the scientific research. Authors who have video or animation files that they wish to submit with their article should send them as a separate file. Reference to the video material should be clearly made in text. This will the modified into a linked to the paper's supplementary information page. All submitted files should be properly labelled so that they directly relate to the video files content. This should be within a maximum size of 50 MB.

Submission check list

The following list will be useful during the final checking of a manuscript prior to sending it to the journal for review. Please consult this Guide for Authors for further details of any item.

- One author has been designated as the corresponding author with contact details:
  - E-mail address.
  - Full postal address.
  - Telephone and fax numbers.
- All necessary files have been sent, and contain:
  - All figures are given separately in PDF, SVG, JPEG of PNG format.
  - Caption for figures is included at the end of the text.
  - All tables (including title, description, footnotes) are included in the text and large tables have been given separately as CSV.
  - The reference list has been given in XML, RIS, zotero.splite or BIB file format.
- Further considerations
  - Abstract does not exceed about 250 words.
  - Manuscript has been 'spell-checked' and 'grammar-checked'.

- References are in the required format.
- All references mentioned in the reference list are cited in the text, and vice versa.
- Bibliographic data for all cited material has been given.
- Permission has been obtained for use of copyrighted material from other sources (including the Web).
- A PDF document generated from the word processor used is given.

After Acceptance

Use of the Digital Object Identifier The Digital Object Identifier (DOI) may be used to cite and link to electronic documents. The DOI consists of a unique alpha-numeric character string which is assigned to a document by the publisher upon the initial electronic publication. The assigned DOI never changes. Therefore, it is an ideal medium for citing a document, particularly 'Articles in press' because they have not yet received their full bibliographic information. When you use a DOI to create links to documents on the web, the DOIs are guaranteed never to change.

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Conference proceedings

6th Mediterranean Neuroscience Society Conference 2017 MALTA MNS2017

Welcome Message

Dear Colleagues,

It is my great pleasure to welcome all delegates on behalf of the Malta Neuroscience Network of the University of Malta to the 6th Mediterranean Neuroscience Society (MNS) Conference 2017, in Malta.

This biennial MNS Conference, unlike most regional events, will bring together neuroscientists from Mediterranean regions and worldwide international experts at the forefront of basic and clinical neuroscience; moreover, it is being held in a vibrant fun location.

The President of MNS, Marc Landry, the Vice-President Liana Fattore, myself and the other members of the scientific committee have worked hard for a very successful event, both scientifically and socially. The MNS2017 programme is exceptional. It includes 8 plenary lectures, the FENS special lecture, more than 60 symposia and hundred of poster presentations which will further stimulating interaction between participants. This 6th MNS Conference will surely be a memorable experience.

The 6th MNS Conference has been organised with the support of the University of Malta, one of Europe's oldest universities in one its most beautiful Mediterranean islands, and Malta Chamber of scientists. We also thank FENS, IBRO and all the other partners for their support.

I very much am looking forward to seeing you at the 6th MNS Conference in Malta!

Giuseppe Di Giovanni
Coordinator of Malta Neuroscience Network
President of the Local Organizing Committee

Mediterranean Neuroscience Society (MNS)

The MNS has been created to support and help strengthen all initiatives that bring together Mediterranean neuroscientists.

The last Mediterranean Conference of Neuroscience was organized in June 2015 in Pula (Italy), four previous successful editions (Montpellier 1997, Marrakech 2006, Alexandria 2009, Istanbul 2012).

These conferences gathered scientists from all Mediterranean countries and offered a rich program with lectures, symposia poster sessions and social events. These meetings have proved to be highly beneficial, not only for the scientific exchanges, but also in terms of training opportunities for students and young researchers.

Research on brain function in health and disease is among the priorities for today's societies, and several indicators put the Mediterranean research area among strategic issues for the European Union (EU).

Many South-North collaborations and networks have emerged in recent years through bilateral and multilateral actions, supported by the EU or by international and national actions, whether for setting up teaching curricula (Tempus programs), or by building human potential (Horizon programs).

Objectives of the MNS

The MNS works towards three main objectives:

- Strengthen exchanges between Mediterranean neuroscientists;
- Promote education in the neurosciences and increase public awareness of progress made;
- Sustain the Mediterranean Neuroscience Conference;
- To reach these objectives, the MNS's policy is to work in close cooperation with existing national and international Neuroscience Societies.

Executive committee

Landry Marc (France)	President
Fattore Liana (Italy)	Vice President
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Main products: Instruments for *In-Vivo* Research focused on Neuroscience
Telephone: +33 442 344 360
Website: www.bioseb.com
Anne Deserve
export@bioseb.com

TSE Systems
Silvia Reinheimer Silvia.Reinheimer@TSE-Systems.com

Stoelting Europe
Odhran Byrne o.byrne@stoeltingeurope.com

Eicom Europe
Richard Martin o.byrne@stoeltingeurope.com

Neuralynx Europe
Emer Murnane o.byrne@stoeltingeurope.com

Ugo Basile S.R.L.
Main products: Instruments for Behavioral Pharmacology
Telephone: +39 0332 744574
Website: www.ugobasile.com
sales@ugobasile.com

FENS
Lars Kristiansen lars.kristiansen@fens.org

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INFORMATION FOR SPEAKERS AND CHAIRPERSONS

The role of the chairpersons is to monitor speaking and discussion times and to lead the discussions. Chairpersons control the switch between presentations.

Use a presentation in the 4:3 format. All speakers must submit their presentations in the Secretary Room, on the ground level of the venue in the Ballroom, at least 2 hours before the start of their session in order to check their slides with the technical staff and upload the presentation onto the network.

Multimedia Considerations and Slide Preparation

Presenters: Make your presentations compatible with on-site audio/visual specifications.

All screens in Malta will be 4:3 widescreen format.

Before Travelling (Recommended)

It is highly recommended to send your presentation by email from the 5th to the 11th of June to: malta.neuroscience@gmail.com SPECIFYING IN THE SUBJECT Symposium number and Symposium presentation (e.g. S33.2 Symposium no. 32 presentation no. 2).

Speakers can upload their presentations at any time from the beginning of the Conference and do not need to wait until the day of presentation. Speakers in morning sessions are strongly advised to pre-load their presentation the day before.

Data presentation

If using a PowerPoint presentation (or any other PC based application), please note you need to bring it on a USB Memory stick and load it on one of the Conference computers in the Secretary Room at least 2 hours before the start of the session.

Please note that the conference computers in the session halls are being supplied with Office 2013.

If combining video films with PowerPoint, please make sure to check it in the session hall where your lecture is taking place during a coffee or lunch break prior to your session, at least 30 minutes before the start of the session - even after checking it in the Speakers' Ready Room.

Alternatively you may supply your own laptop computer. In such a case please confirm that it has a VGA socket for external signal and **come to check it first in** Secretary Room as soon as you arrive and later on in the session hall where your lecture is taking place during the coffee or lunch break prior to your session, at least 30 minutes before the start of the session.

Important note for Macintosh users

In order to use MAC presentations on a PC compatible computer please note that you need to prepare it according to the instructions below, before bringing it to the Secretary Room:

- Use a common font, such as Arial, Times New Roman, Verdana etc. (special fonts might be changed to a default font on a PowerPoint based PC).
- Insert pictures as JPG files (and not TIF, PNG or PICT - these images will not be visible on a PowerPoint based PC).

Alternatively you may use your own Macintosh laptop computer. In such a case please confirm you provide it with a **VGA adaptor** for external signal, advise the operators in the Secretary Room about it as soon as you arrive and later on test it in the session hall where your lecture is taking place during the coffee or lunch break prior to your session, at least 30 minutes before the start of the session.

POSTER INFORMATION

Poster Presentations

Whole-day poster presentations take place in the Poster Area of the exhibition hall in front to the main restaurant from Monday through Thursday, June 12–15. Authors are requested to be in attendance at their poster for discussion, as scheduled below:

Monday, June 12–Thursday, June 15
08:00–16:15 **Poster session**
12:30–14:00 **All poster authors in attendance**

Please find your board number by locating your abstract on the programme book. You should display your poster on the board number assigned to you.

Poster boards are 2 meters high and 1 meter wide (portrait format).

Posters can be affixed by double-sided adhesive tape, available at the Poster Assistance desk onsite. Posters should be mounted at 08:00 am. Removal: Posters from the day sessions must be removed imperatively at the end of the day of presentation. Please respect this removal so that the following poster presenters can mount their material.

The organisers cannot be responsible for posters not removed by the above stated time.

PROGRAMME
Monday, 12th June 2017

07.30–19.00
Registration

08.30–09.00
MN 2017 Opening Ceremony Marc Landry, MNS President, Giuseppe Di Giovanni, President of the 2017 MNS Meeting, the Rector of the University of Malta, Prof. Alfred J Vella and the President of Malta, Marie Louise Coleiro Preca

09.00–10.00
PLENARY LECTURE
Introducer: *L. Fattore (IT)* **Room: Ballroom**
The EMBO Plenary Lecture

HOW THE IMMUNE SYSTEM AFFECTS SYNAPTIC FUNCTION
Michela Matteoli (IT)

10.00–10.30
Coffee break and Posters

10.30–12.15
Parallel Symposia

S1 **Room: Ballroom**
NEW DIRECTIONS IN SEROTONIN RECEPTOR RESEARCH AND THERAPEUTICS
Chair: Umberto Spampinato, FR

S1.1 Umberto Spampinato An ignored neural receptor: the 5-HT2B receptor strategies for therapeutics in neuropsychiatric disorders
S1.2 Haddjeri Nasser 5-HT_3 and 5-HT_7 receptor antagonism potentiates antidepressant response
S1.3 Malgorzata Filip 5-HT_{2C} receptors as a therapeutic strategy in substance use disorders: focus on cocaine
S1.4 Francesco Artigas Perez Improving speed and efficacy of antidepressant drugs: focus on 5-HT_{1A} and 5-HT_3 receptors

S2 **Room: Clermont Suite**
STIMULATING THE TOURETTE BRAIN – OBTAINING THERAPEUTIC RELIEF AND MECHANISTIC INSIGHT
Chairs: Christine Winter, DE and Laura Pozzi, SE

S2.1 Christine Winter Tourette Syndrome in humans and rats: Brain stimulation techniques in the context of repetitive disorders
S2.2 Henriette Callesen Brain stimulation techniques in the context of repetitive disorders
S2.3 Laura Pozzi Employing optogenetics to elucidate the function of parvalbumin (PV+) interneurons in the context of Tourette Syndrome
S2.4 Alberto Priori Adaptive deep brain stimulation (aDBS) in Tourette syndrome

S3 **Room: Marie Louise 1**
PRECLINICAL AND TRANSLATIONAL APPROACHES FOR THE INVESTIGATION OF DRUG ADDICTION
Chairs: Marcello Solinas, FR and Roberto Ciccocioppo, IT

S3.1 Marcello Solinas Persistent neuroadaptations following extensive cocaine self-administration and strategies to boost recovery processes
S3.2 Christelle Baunez Surgical strategy for addiction based on rats and monkeys studies using deep brain stimulation applied at the level of the subthalamic nucleus
S3.3 Roberto Ciccocioppo Translational strategies for the development of new medications to treat drug addiction
S3.4 Rafael Maldonado Role of the endocannabinoid system in food-induced addictive-like behaviour

S4 **Room: Reading Room**
THE MULTI-OSCILLATORY CIRCADIAN NETWORK: A KEY FOR PHYSIOLOGICAL PROCESSES AND HEALTH
Chair: Paul Pévet, FR

S4.1 Andries Kalsbeek Glucose regulation: a regulatory feedback network of periphery and brain
S4.2 Paul Pévet The multi-oscillatory circadian network: A key for physiological processes and health
S4.3 Challet Etienne Rodents models to study the effect on humans of disturbed circadian rhythms and metabolism
S4.4 El Allali Khalid Synchronisation of clocks: the ambient temperature as the forgotten "zeitgeber" in mammals
S4.5 Marie-Paule Felder-Schmittbuhl Clocks within the eyes: a key for a well-functioning retina

S4.6 Johanna H. Meijer Role of the Behavioural feedback on the clock functioning

S5 Room: Carlson Suite
GPCR HETERORECEPTOR COMPLEXES AND THEIR ADAPTOR PROTEINS IN PARKINSON'S DISEASE AND SCHIZOPHRENIA
Chair: Kjell Fuxe, SE

S5.1 Kjell Fuxe GPCR heteroreceptor complexes and their adaptor proteins give new integrative mechanisms that may go wrong in Parkinson's disease and schizophrenia
S5.2 Fang Liu D2-DISC1 protein complexes and their relevance for schizophrenia
S5.3 Rafael Franco Role of the endocannabinoid system in food-induced addictive-like behaviour
S5.4 Per Svenningsson The cellular scaffold protein p11 strongly modulates the therapeutic effects of L-DOPA in PD. Focal inhibition of p11 as a novel therapeutic target
S5.5 Dasiel Oscar Borroto Escuela A2A heteroreceptor complexes as therapeutic targets in Parkinson's disease

S6 Room: Marie Louise 2
PAIN AND COMORBID MENTAL DISORDERS
Chair: Marc Landry, FR

S6.1 Frank Porreca Reward and motivation in pain and pain relief
S6.2 Poisbeau Pierrick Long-term consequences of perinatal stress on the nociceptive system and pain controls
S6.3 Bouchatta Otmane Alterations of Pain Response in a pharmacological model of an ADHD mouse model
S6.4 David P. Finn Neural sites and mechanisms mediating reciprocal interactions between pain and negative affect
S6.5 Olivier Roca-Lapirot Acquisition of analgesic properties of the CCK system within the amygdala after inflammation

12.30–14.00
Lunch
P12.1–P12.26 Poster Authors in Attendance

14.00–15.45
Parallel Symposia

S7 Room: Ballroom
5-HT$_{2C}$ RECEPTORS: WIDESPREAD CONTROL OF NEUROBIOLOGICAL NETWORKS: I
Chair: Giuseppe Di Giovanni, MT

S7.1 Ponimaskin Evgeni Comparative analysis on 5-HT$_{2C}$, 5-HT$_{1A}$ and 5-HT$_7$ receptors on their role in anxiety and depression
S7.2 Lora Heisler Identification of the subset of 5-HT$_{2C}$Rs that control appetite and improve obesity and type 2 diabetes
S7.3 Kathryn Cunningham 5-HT$_{2C}$R Positive Allosteric Modulators: Potential for Therapeutics in Addictive Disorders

S8 Room: Clermont Suite
NEUROINFLAMMATION: DISEASE MODELS, MOLECULAR AND CELLULAR MARKERS, *IN VIVO* MONITORING
Chairs: Klaus G. Petry, FR and Sylvie Chalon, FR

S8.1 Klaus G. Petry The identification of peptide biomarkers mimicking the complexity of protein interactions at the blood brain barrier in experimental Multiple Sclerosis
S8.2 Sylvie Chalon PET imaging of TSPO to monitor microglia activation and their relevance to disease severity progression in models of Subarachnoid Hemorrhage, Alzheimer and Parkinson diseases
S8.3 Anna Planas The monocytes recruitment in stroke affection and their phenotype modulation in CNS repair
S8.4 Mario Quarantelli MRI modalities to investigate neuroinflammation in experimental and clinical studies

S9 Room: Carlson Suite
TRANSCRANIAL MAGNETIC STIMULATION: RE-WIRING THE ADDICTED BRAIN
Chair: Marco Diana, IT

S9.1 Marco Diana Exploiting the hypodopaminergic state with TMS in addicts: preliminary observations
S9.2 Markus Heilig Evaluating deep TMS (dTMS) targeting the insula as a potential treatment for alcohol dependence: Design issues and interim results
S9.3 Abraham Zangen Electromagnetic stimulation in the study and treatment of addiction: From animal models to human applications
S9.4 Antonello Bonci From optogenetics to a novel clinical treatment against cocaine use disorders

S10 Room: Reading Room
OXIDATIVE STRESS IMPLICATION IN NEUROPATHOLOGY
Chairs: Amira Zaky, EG and Nicola Mercuri, IT

S10.1 Amira Zaky APE1 paradox in neuropathic pain management: novel insight on its role as a regulator for microRNAs

S10.2 Youssef Anouar Selenoproteins, oxidative stress and Parkinson's disease
S10.3 Nicola Biagio Mercuri Dopaminergic dysregulation in animal models of degenerative diseases

S11 Room: Marie Louise 1
NEUROCYTOSKELETON FUNCTIONS AND DYSFUNCTIONS IN NEURODEGENERATIVE AND PSYCHIATRIC DISEASES
Chairs: Annie Andrieux, FR and Marie Jo Mountin, FR

S11.1 C. Laura Sayas Casanova Crosstalk between microtubule-associated proteins and +TIPs in neuronal cells: possible implications in neurodegeneration
S11.2 Marie-Jo Moutin Tubulin modifying enzymes in neurodegenerative contexts
S11.3 Annie Andrieux Positive effects of cytoskeleton-related drugs on cognitive abilities in an animal model of psychiatric diseases
S11.4 Graziella Cappelletti Microtubule dysfunction in animal models of Parkinson disease
S11.5 Márcia Liz The cytoskeleton as a novel therapeutic target for old neurodegenerative disorders

S12 Room: Marie Louise 2
GENETIC AND EPIGENETIC REGULATION OF STRESS SENSITIVITY FROM MOUSE TO MAN: RELEVANCE TO STRESS-RELATED DISORDERS
Chair: Nicolas Singewald, AT

S12.1 Luisa Pinto Genetic and epigenetic factors that modulate stress-induced neuro- and glio-plasticity: relevance to depression
S12.2 Catherine Belzung Epigenetic signature of chronic stress and antidepressant response
S12.3 Nicolas Singewald (Epi)genetic regulation of stress and fear sensitivity in mouse models of anxiety
S12.4 Andrew Holmes Genetic moderation of stress systems influencing fear extinction
S12.5 John Cryan Microbial genes, brain and behaviour: regulation of stress susceptibility by the microbiome

15.45–16.15
Coffee break and Posters

Parallel Symposia
S13 Room: Ballroom
GENETIC AND EPIGENETIC REGULATION OF STRESS SENSITIVITY FROM MOUSE TO MAN: RELEVANCE TO STRESS-RELATED DISORDERS
Chairs: Giuseppe Di Giovanni, MT and Umberto Spampinato, FR

S13.1 Alfredo Meneses Role of 5-HT_2 receptors in cognition
S13.2 Umberto Spampinato 5-HT_{2C} receptors: a key pharmacological target to control DA neuron activity and DA-dependent behaviors: focus on cocaine
S13.3 Cristiano Bombardi 5-HT_{2A} and 5-HT_{2C}R expression profile in young and adult GAERS, NEC and WISTAR rats

S14 Room: Marie Louise 2
THE SICK BRAIN
Chair: Ana María Sánchez-Pérez, ES

S14.1 Peter McCormick G-protein coupled receptor heterodimers: powerful drug targets for the treatment of disease
S14.2 Ludvic Zrinzo A randomised controlled trial of Deep Brain Stimulation in severe refractory obsessive compulsive disorder
S14.3 Ana María Sánchez-Pérez Insulin/IGF-1 signalling in pathological situationsy
S14.4 Mohamed Najimi Neurobehavioural effects of developmental toxicity of heavy metals

S15 Room: Reading Room
NOVEL PSYCHOACTIVE SUBSTANCES: BEHAVIOURAL, NEUROCHEMICAL, MOLECULAR EFFECTS AND UNDERLYING MECHANISMS
Chair: Maria Antonietta De Luca, IT

S15.1 Michael Baumann Neuropharmacology of newly-emerging synthetic stimulants: cathinones and beyond
S15.2 Liana Fattore Effect of the ketamine-like compound methoxetamine on brain reward processing and emotional states
S15.3 Cristiano Chiamulera Ketamine-induced neuroplasticity after acute vs. chronic self-administration in rats: how to keep apart "Dr Jekyll from Mr Hyde"
S15.4 Colin Davidson Potential clinical use of some legal highs
S15.5 Gaetano Di Chiara Third generation synthetic cannabinoids

S16 Room: Marie Louise 1
NEW PERSPECTIVES IN ANIMAL MODELS OF DEPRESSION: PHARMACOLOGICAL, ANATOMICAL, ENDOCRINE AND BEHAVIORAL APPROACHES
Chairs: Mercè Correa, ES and John D. Salamone, USA

S16.1 Wolfgang Hauber Cognitive flexibility: Corticostriatal circuits and neurochemical modulation in rats

S16.2 John Salamone Effort-related decision making in human depression and animal models: Distinct roles for different monoamines
S16.3 Mercè Correa Impact of exercise on the dopaminergic system and on selection of active vs. passive sources of reinforcement: implications for the treatment of anergia in depression
S16.4 Antonio Armario Chronic unpredictable stress as an animal model of depression: new data and new perspectives

S17 Room: Marie Louise 2
NEW PERSPECTIVES IN ANIMAL MODELS OF DEPRESSION: PHARMACOLOGICAL, ANATOMICAL, ENDOCRINE AND BEHAVIORAL APPROACHES
Chair: Ruben J. Cauchi, MT

S17.1 Cinzia Cagnoli The role of survival motor neuron proteins (FL-SMN and a-SMN) on axonal function and motor neuron phenotype in mouse and cell models of spinal muscular atrophy
S17.2 Ruben Cauchi Drosophila models of spinal muscular atrophy to untangle the function of the survival motor neuron (SMN) complex in the neuromuscular system
S17.3 Remy Bordonne Characterization of survival motor neuron (SMN)-dependent pathways using the S. pombe model organism
S17.4 Elia Di Schiavi Identification of neuroprotective molecules for spinal muscular atrophy using the C. elegans animal model

S18 Room: Reading Room
MOLECULAR INSIGHT INTO PAIN PERCEPTION
Chairs: Mark Landry, FR and Jacques Noël, FR

S18.1 Ana Gomis Piezo2 and proprioception
S18.2 Xavier Gasull Regulation of TRESK K2P channels and role on pain sensation
S18.3 Radwani Houda Switch from excitatory to inhibitory modulation by group I metabotropic glutamate receptors of nociceptive transmission in a model of inflammatory pain
S18.4 Jacques Noël Non-acidic activation of pain-related acid-sensing ion channel 3 by lipids

19.00–20.00
FENS SPECIAL LECTURE
Introducer: *M. Landry (FR)* **Room: Ballroom**

FENS Federation of European Neuroscience Societies

KAINATE RECEPTORS IN MOOD DISORDERS
Juan Lerma (ES)

PLENARY LECTURE
Introducer: *G. Di Giovanni (MT)*
Room: Ballroom

UNIVERSITY OF MALTA
L-Università ta' Malta

The University of Malta Plenary Lecture
FROM MIRROR NEURON TO MIRROR BRAIN
Giacomo Rizzolatti (IT/MT)

20.00–21.30
Welcome Cocktail

Tuesday, 13th June 2017

07.30–19.00
Registration

08.30–09.00
Posters on display

09.00–10.00
PLENARY LECTURE
Introducer: ***G. Di Giovanni (MT)***
Room: Ballroom

UNIVERSITY OF MALTA
L-Università ta' Malta

The University of Malta Plenary Lecture
CELLULAR AND NETWORK DYNAMICS OF EEG WAVES OF HEALTH AND DISEASE STATES
Vincenzo Crunelli (UK/MT)

10.00–10.30
Coffee break and Posters

10.30–12.15
Parallel Symposia

S19 **Room: Ballroom**
NEW INSIGHTS ON FUNCTIONAL INTEGRATION IN THE CORTICO-THALAMO-CORTICAL CIRCUIT
Chair: Vincenzo Crunelli, UK/MT

S19.1 Alexander Groh Thalamocortical interactions during sensory processing in the mammalian brain
S19.2 Anita Luthi Modulation of Cav3-channel- dependent thalamic rhythmogenesis through corticoreticular synaptic plasticity
S19.3 Magor Lorincz Rapid brain state dependent modulation of spontaneous and sensory evoked activity in the lateral geniculate nucleus of awake mice

S20 **Room: Carlson Suite**
NEW INSIGHTS INTO BASAL GANGLIA CIRCUITRY: IMPLICATIONS FOR UNDERSTANDING AND TREATING BASAL GANGLIA DISORDERS
Chairs: Suzanne N. Haber, USA and Hagai Bergman, IL

S20.1 Yasin Temel Ultra-high field visualization of functional connections between the pallidal complex, subthalamic nucleus and substantia nigra
S20.2 Suzanne Haber Direct and indirect cortical influence on dopamine neurons
S20.3 Hagai Bergman The subthalamic nucleus and the striatum – driving force vs. fine tuning
S20.4 Benazzouz Abdelhamid The neurophysiological substrate of non-motor disorders in Parkinson's disease

S21 **Room: Marie Louise 1**
Cannabinoid regulation of the mesocorticolimbic dopamine system: Implications for neuropsychiatric disorders
Chair: Steven Laviolette, CA

S21.1 Steven Laviolette Understanding the differential effects of THC and cannabidiol on mesolimbic dopamine function: Implications for the etiology and treatment of schizophrenia
S21.2 Styliani Vlachou To please or not to please? The endocannabinoid system and its involvement in reward and addiction with a focus on intracranial self-stimulation studies
S21.3 Maria Antonietta De Luca Influence of JWH-018 repeated administration on the responsiveness of mesolimbic and mesocortical dopamine transmission to rewarding stimuli
S21.4 Aviv Weinstein The effects of synthetic cannabinoids on executive function and related brain activity in fMRI

S22 **Room: Marie Louise 2**
NEUROLOGICAL MECHANISMS OF THE IMPACT OF STRESS ON MEMORY AND EMOTIONALITY IN HEALTH AND DISEASE: INSIGHTS FROM ANIMAL AND CLINICAL STUDIES
Chairs: Patrizia Campolongo, IT and Maria Morena, CA

S22.1 Benno Roozendaal Stress and emotional arousal effects on memory
S22.2 Maria Morena Modulation of cognition and emotion by the endocannabinoid signaling is dependent on stress and arousal state
S22.3 Gustav Schelling Glucocorticoids and endocannabinoids: biomarkers and therapeutics for traumatic memories and stress-related disorders – or nothing at all?
S22.4 Gina L Quirarte The role of glucocorticoids in the striatum on emotional memory

S22.5 Dominique de Quervain Stress, genes and glucocorticoids: clinical implications

S23 Room: Clermont Suite
NEW ENDEAVOURS FOR AN ANCIENT STRUCTURE: NOVEL ROLES FOR THE CEREBELLUM IN PHYSIOLOGY AND PATHOLOGY
Chairs: Daniela Carulli, IT and Marta Asunta Miquel Salgado-Araujo (ES)

S23.1 Laura Petrosini Viewing the personality traits through a cerebellar lens
S23.2 Robert Barton Cerebella comes to the ball: cognitive evolution and the cerebellum
S23.3 Daniela Carulli Purkinje cells lacking PTEN: the autistic cerebellum
S23.4 Marta Miquel The cerebellum's roles in addiction. Have we been ignoring the elephant in the room?
S23.5 Chris De Zeeuw Why we dance better than robots

S24 Room: Reading Room
THE EVOLUTION OF NEURONAL NETWORKS: INSECTS IN NEUROBIOLOGICAL STUDIES
Chairs: Martin Giurfa, FR and Hans-Joachim Pflueger, DE

S24.1 Hans-Joachim Pflueger On the control of locomotion in insects and the importance of neuromodulati
S24.2 Martin Giurfa On learning and memory formation in bees
S24.3 Silke Sachse Elucidating olfactory coding mechanisms within and beyond the Drosophila antennal lobe
S24.4 Frederic Libersat On behavioral and neural manipulations of insects by parasitic wasps

12.30–14.00
Lunch
P13.1–P13.27 Poster Authors in Attendance

14.00–15.45
Parallel Symposia

S25 Room: Marie Louise 1
BRIDGING THE GAP: NOVEL DEVELOPMENTS IN THE TREATMENT OF EPILEPSY
Chair: Janet Mifsud, MT

S25.1 Janet Mifsud The role of European funded research in bridging the gap in epilepsy
S25.2 Christian Saliba Pharmacogenetics of lamotrigine metabolism in paediatric populations
S25.3 Sanjay Sisodiya Precision medicine in the epilepsies
S25.4 Renzo Guerrini The genetic epileptic encephalopathies: from molecular diagnosis to management strategies

S26 CANCELLED Room: Carlson Suite
NEURONAL VULNERABILITY AND SYNUCLEINOPATHIES – NEW INSIGHTS IN PARKINSON'S DISEASE
Chair: Jochen Roeper, DE

S26.1 Ledia F. Hernandez Habitual and goal directed behavior in a Parkinson disease model
S26.2 Jochen Roeper Mechanisms of acquired vulnerability of substantia nigra dopamine neurons in alpha-synuclein models of Parkinson's disease

S27 Room: Ballroom
DEVELOPMENT OF CANNABINOID-BASED THERAPIES TO PRESERVE NEURONS AGAINST ACUTE OR CHRONIC DAMAGE
Chair: Javier Fernández-Ruiz, ES

S27.1 Mauro Maccarrone Cannabinoids and epigentic regulation in neurodegenerative disorders
S27.2 Ester Aso Cannabinoids for the treatment of Alzheimer's disease
S27.3 Javier Fernández-Ruiz The endocannabinoid signaling provides druggable targets for neuroprotection: an overview
S27.4 Onintza Sagredo Preclinical and clinical studies with cannabinoids in Huntington's disease
S27.5 Antonio Pisani Cannabinoids for the treatment of Parkinson's disease

S28 Room: Clermont Suite
FUNCTIONAL CONNECTIVITY STUDIES ON MOTOR AND LANGUAGE LEARNING WITH HEALTHY AND BRAIN DAMAGED ADULTS
Chair: Ana Inés Ansaldo, CA

S28.1 Benali Habib Functional Connectivity in fMRI: An Overview of Connectivity Methods
S28.2 Yves Joanette Age-related Changes in Functional Connectivity for Word Production
S28.3 Julien Doyon Neural Network Changes Mediating Motor Skill Learning and Consolidation
S28.4 Ana Inés Ansaldo Executive control Functional Networks in Bilinguals and Monolinguals

S28.5 Olga Dragoy Reorganization of Language Networks in Individuals with Aphasia

S29 Room: Marie Louise 2
SUBSTANCES OF ABUSE DURING PREGNANCY: IS IT ALL PLEASURE?
Chair: Carla Cannizzaro, IT

S29.1 Miriam Melis Maternal Δ9-THC: functional consequences on offspring mesolimbic dopamine transmission
S29.2 Carla Cannizzaro Long-term trajectories in alcohol consumption from the mother to the offspring: a "thin red line"
S29.3 Tania Marcourakis Environmental tobacco smoke in the early postnatal period – effects on brain glucose metabolism and endocannabinoid system
S29.4 Olivier Manzoni In-utero cannabis

S30 Room: Reading Room
PROGRAMMING OF OFFSPRING DEVELOPMENT BY MATERNAL ENVIRONMENT
Chair: Muriel Koehl, FR

S30.1 Aniko Korosi Early nutritional intervention prevents the early-life stress induced cognitive impairments
S30.2 Fotini Stylianopoulou Denial of the reward of maternal contact affects social behaviour and the serotonergic system in the adult rat brain
S30.3 Muriel Koehl Prenatal stress and programming of stress-induced emotional and memory states
S30.4 Chris Murgatroyd Transgenerational accumulation of impairments in maternal behaviour following postnatal social stress

15.45–16.15
Coffee break and Posters

Parallel Symposia
S31 Room: Reading Room
A NOVEL WAY TO APPROACH NEUROPSYCHIATRIC DISORDERS: INVESTIGATING NEW MOLECULAR PATHWAYS
Chair: Luigia Trabace, IT

S31.1 Maria Grazia Morgese Soluble beta amyloid: a bridge between Alzheimer's disease and depression?
S31.2 Stefania Schiavone Detrimental and beneficial effects of reactive oxygen species in psychiatric disorders: toward novel pharmacological targets
S31.3 Filippo Caraci Deficit of neurotrophin signaling in Alzheimer's disease: searching for new pharmacological targets
S31.4 Brian Harvey Investigating immune-inflammatory targets in translational animal models of schizophrenia and their response to N-acetyl cysteine
S31.5 Martien Kas Novel approaches to link quantitative biology to neuropsychiatry

S32 Room: Carlson Suite
EMERGING VIEWS ON DOPAMINE SIGNALING AND WHAT CAN WE LEARN FROM STATE OF THE ART TECHNOLOGIES IN ANIMAL MODELS OF PD
Chair: Rosario Moratalla, SP

S32.1 Ledia F. Hernandez Impact of optogenetically driven striatal projection neurons in L-DOPA-induced dyskinesias
S32.2 Rosario Moratalla Synaptic plasticity changes after L-DOPA in the lesioned striatum
S32.3 Gilberto Fisone Non-motor symptoms in Parkinson's disease: Modeling and mechanisms
S32.4 Raffaella Tonini Integrated dopaminergic and serotonergic signaling at glutamatergic striatal synapses

S33 Room: Ballroom
DEVELOPING NOVEL TOOLS FOR ENDOCANNABINOID RESEARCH
Chairs: Mauro Maccarrone, IT and Stefanie Butini, IT

S33.1 Mauro Maccarrone A modern view of the endocannabinoid system and need for selective drugs
S33.2 Mario van der Stelt New selective drugs for DAG lipases
S33.3 Uwe Grether New selective drugs for cannabinoid receptors
S33.4 Stefania Butini Development of novel enzyme inhibitors of the endocannabinoids' catabolism for the treatment of epilepsy and multiple sclerosis
S33.5 Jürg Gertsch New selective drugs for endocannabinoid transport

S34 Room: Marie Louise 2
NOVEL PERSPECTIVES IN VISUOMOTOR LEARNING AND OBJECT REPRESENTATION FOR ACTION AND PERCEPTION
Chairs: Annalisa Bosco, IT and Luca Turella, IT

S34.1 Roy Mukamel Neural networks underlying learning of motor skills from others
S34.2 Luca Turella Neural correlates of object encoding for goal-related actions
S34.3 Annalisa Bosco The influence of different predictive conditions of subsequent action on object size perception

S34.4 Boujraf Saïd Correlative study of impairment of learning process using visuo-motor association and MRI data in Parkinson patient without comorbidities

S35 Room: Marie Louise 1
SENSORIMOTOR ACTIVITY ASSESSMENT IN HEALTHY AND PATHOLOGICAL CONDITIONS
Chairs: Kenneth P. Camilleri, MT, Tracey Cassar, MT and Owen Falzon, MT

S35.1 Geert Verheyden Behavioural and neural correlates of somatosensory deficits in arm and hand post stroke
S35.2 Andrei Agius Anastasi Neurorehabilitation technologies for stroke
S35.3 Lisa Tedesco Triccas Exploring cortical activity measured by electroencephalography of upper limb sensorimotor impairments and recovery after stroke
S35.4 Pietro Avanzini The recruitment of sensorimotor system during action execution and observation as revealed by electrophysiological investigations
S35.5 Maddalena Fabbri Destro The tight link between action execution and recognition in children with autism: echoing an out-of-tune chord?

S36 Room: Clermont Suite
THE MIND IN THE MATRIX: EXTRACELLULAR MATRIX MOLECULES AS CRITICAL FACTORS FOR PSYCHIATRIC
Chair: Constanze Seidenbecher, DE

S36.1 Sabine Spijker Caught in the web of depression
S36.2 Sabina Berretta Not only neurons: Extracellular matrix abnormalities in major psychoses
S36.3 Chavis Pascale Developmental endophenotypes in the prefrontal cortex are revealed by the risk factor reelin
S36.4 Alexander Dityatev Extracellular matrix and neuroplasticity in health and disease

18.00–19.00
Minisymposia

MS1 Room: Ballroom
Chair: Mark Wall, UK

MS1.1 Mark Wall Adenosine plays a key role in controlling Ca^{2+} free seizures
MS1.2 Gabriele Deidda Modulation of intracellular chloride to regulate synaptic plasticity and rescue cognitive functions in down syndrome
MS1.3 Sara Anna Bonini Maternal immune activation and abnormal brain development new insights on neurodevelopmental disorders pathogenesis

MS2 Room: Clermont Suite
Chair: Goran Simic, HR

MS2.1 Akash Gautam Study of Arc-mediated changes in the morphology of dendritic spines during amnesia and its restoration
MS2.2 Josanne Aquilina Deep brain stimulation as a new service in Malta through cross border collaboration - audit of the first five years
MS2.3 Goran Simic Lymphatic and glymphatic systems as origin points for Alzheimer's disease

MS3 Room: Marie Louise 1
Chair: Adrianna Mendrek, CA

MS3.1 Nicola Simola Effects of corticosterone on the initiation of 50 kHz ultrasonic vocalizations and on calling stimulated by social contacts and amphetamine
MS3.2 Claudio D'Addario Synergistic interactions between opioid and cannabinoid systems in obesity
MS3.3 Adrianna Mendrek Effects of nicotine on cognitive function across the menstrual cycle in non-smoking women

MS4 Room: Marie Louise 2
Chair: Nikolaos Kokras, CA

MS4.1 Yassine Ait Bali Effect of Glyphoaste subchronic and chronic exposure in juvenile mice - behavioral and immunohistological study
MS4.2 Michael Galea The Relationship of Personality, Spirituality and Posttraumatic Growth to Subjective Wellbeing
MS4.3 Nikolaos Kokras Sex differences in experimental studies of depression: how can clinical research benefit

MS5 Room: Carlson Suite
Chair: Tiziana M Florio, IT

MS5.1 Carmen de la Fuente Barrigon Dopamine metabolism analysis in different cellular models of Parkinson's disease
MS5.2 Michelle Briffa Extracts from two ubiquitous Mediterranean plants ameliorate cellular and animal models of neurodegenerative proteinopathies
MS5.3 Tiziana M Florio The 6-hydroxydopamine Hemiparkinsonian Rat Model: Evidence of Early Stage Degeneration of the Nigrostriatal Pathway

MS6 Room: Reading Room
Chair: Meha Fatima Aftab, PK

MS6.1 Francesca Calabrese TPH2-deficient rats show alterations of neuroplastic mechanisms in basal condition and after an acute stress
MS6.2 Meha Fatima Aftab Isatin derivative inhibits neuronal pathology induced by reactive compounds present in processed sugars

MS6.3 María Belén Ruíz-Roso Guerra Effects of DHA low in phytanic acid (OPHY3) in memory and biomarkers of neurodegeneration in hippocampus of APOE– mice fed a high fat diet

19.00–20.00
PLENARY LECTURE
Introducer: *L. Fattore (IT)*

Room: Ballroom

THE ENDOCANNABINOIDS: LOOKING BACK AND AHEAD
Raphael Mechoulam (IL)

20.00–21.00
MNS General Assembly

Wednesday, 14th June 2017

07.30–19.00
Registration

08.30–09.00
Posters on display

09.00–10.00
PLENARY LECTURE
Introducer: ***M. Landry (FR)*** **Room: Ballroom**

The IBRO Plenary Lecture
NEURON-GLIA METABOLIC COUPLING: ROLE IN NEURONAL PLASTICITY AND NEUROPROTECTION
Pierre Magistretti (CH)

10.00–10.30
Coffee break and Posters

10.30–12.15
Parallel Symposia

S37 **Room: Ballroom**
GLIAL PERSPECTIVES IN BRAIN DAMAGE AND REPAIR
Chairs: Lucio Annunziato, IT and Alexei Verkhratsky, ES

S37.1 Mami Noda Role of glial cells in the relationship between thyroid dysfunction and mental disorders
S37.2 Francesca Boscia Emerging role of the sodium calcium exchanger NCX3 in oligodendrocytes during CNS demyelination and remyelination
S37.3 Alexei Verkhratsky Complex and differential glial responses in Alzheimer's disease and ageing
S37.4 Maria Pia Abbracchio CNS remyelination as a novel reparative approach to neurodegenerative diseases – the role of the P2Y-like receptor GPR17

S38 **Room: Marie Louise 1**
NEUROINFLAMMATION AND MITOCHONDRIAL DYSFUNCTION IN PARKINSON'S DISEASE
Chair: Nicola Simola, IT

S38.1 Antonella Scorziello NCX1 and NCX3 as potential players in mitochondrial promoted neuroinflammation in *in vitro* and *in vivo* models of PD
S38.2 Gian Carlo Bellenchi miR-34b/c enhances mesencephalic dopaminergic neurons differentiation by negatively modulating the Wnt signalling
S38.3 Gyorgy Szabadkai Interaction of mitochondria with the nucleus and endoplasmic reticulum in neurodegeneration
S38.4 Gemma Navarro Brugal Anti-inflammatory and neuroprotective effects of cannabinoids in parkinsonian conditions

S39 **Room: Clermont Suite**
NEW INSIGHTS ON THE ENDOCANNABINOID SYSTEM: FROM THE MOLECULAR MECHANISM TO PHARMACOLOGICAL APPROACHES
Chairs: Roberto Colangeli, CA and Gordon Campbell Teskey, CA, IT

S39.1 Gordon Campbell Teskey Endocannabinoid involvement in motor map expression and motor behaviour
S39.2 Roger Thompson Regulation of synaptic efficacy by anandamide transport through pannexin-1
S39.3 Roberto Colangeli Epilepsy and comorbidities: involvement of the endocannabinoid system

S40 **Room: Reading Room**
TARGETING NEUROSTEROIDOGENESIS FOR THE TREATMENT OF NEURODEGENERATIVE AND NEUROPSYCHIATRIC DISEASES
Chair: Roberto Frau, IT

S40.1 Silvia Fanni 5α-reductase inhibition as a possible treatment for dyskinesias in PD
S40.2 Therese Di Paolo The 5α-reductase inhibitor Dutasteride but not Finasteride protects dopamine neurons in the MPTP mouse model of PD
S40.3 Roberto Frau Sleep deprivation induces manic-like behaviours through alterations in neurosteroidogenesis
S40.4 Silvia Giatti Effects of Finasteride in the Nervous System: Focus on Neuroactive Steroids
S40.5 Simona Scheggi Unravelling the molecular mechanism governing the antipsychotic-like effects of finasteride

S41 **Room: Marie Louise 2**
APPLICATION OF T-PATTERN DETECTION AND ANALYSIS (TPA) FOR THE DISCOVERY OF HIDDEN FEATURES OF HUMAN AND ANIMAL BEHAVIOUR
Chairs: Maurizio Casarrubea, IT and Magnus S. Magnusson, IS

S41.1 Marta Castañer Integrating categorical and continuous data to analyse the effects of a varied exercise workout program in adult women

S41.2 Magnus Magnusson From sequential analysis to symmetry and self-similarity: detecting recurrent hierarchical structured temporal clusters in behaviour
S41.3 M. Teresa Anguera Application of T-pattern detection and analysis (TPA) for the discovery of hidden features of human and animal behaviour
S41.4 Maurizio Casarrubea T-pattern analysis in the study of rodent behaviour. Methodological and experimental highlightss
S41.5 Jonsson Gudberg K. Expanding the study of internet gambling behaviour: patterns and trends within the Icelandic Lottery and Sports Betting Platform

S42 **Room: Carlson Suite**
EXPRESSION AND INHIBITION OF CONDITIONED FEAR
Chair: Markus Fendt, DE

S42.1 Raphael Lamprecht The role of actin cytoskeleton and its regulatory proteins in constraining fear memory formation in amygdala
S42.2 Thomas Endres Impact of BDNF/TrkB-signaling on fear expression and fear inhibition
S42.3 Radwa Khalil Learning categorization of fear and safety learning through experimental and computational eyes
S42.4 Ingrid Ehrlich Amygdala circuits and mechanisms that control of acquired fear and its extinction
S42.5 Marianne Ronovsky miRNA and learned safety

12.30–14.00
Lunch
P14.1–P14.24 Poster Authors in Attendance

14.00–15.45
Workshops – Networking – Transversal Symposium

S43 **Room: Ballroom**
TRANSVERSAL SYMPOSIUM: INITIATIVES FOR EURO-MEDITERRANEAN COLLABORATIONS – ROUND TABLE
Chairs: Demian Battaglia, FR and Mohammad Herzallah, USA

Mohammad Herzallah
Ahmed El Hady
David Hansel
Marc Landry
Driss Boussaoud

WS1 – Workshop **Room: Marie Louise 1**
HOW TO GET A PAPER PUBLISHED, READ & CITED ELSEVIER/XJENZA
Shamus O'Reilly
Natalie Farra
Ryan Scicluna
Vincenzo Crunelli
Bruno Frenguelli
Giuseppe Di Giovanni
David Magri

WS2 – Workshop
Room: Marie Louise 2
PUBLIC ENGAGEMENT IN SCIENCE
Edward Duca

WS3 – Workshop
Room: Reading Room
STATISTICAL MODELING
Liberato Camilleri

WS4 – The Matchmaking Event
Room: Carlson Suite
LOOKING FOR YOUR PARTNERS IN RESEARCH

15.45–16.15
Coffee break and Posters

Parallel Symposia
S44 **Room: Carlson Suite**
INSIGHTS ON THALAMIC FUNCTION: A VIEW FROM NEUROPSYCHIATRIC DISORDERS
Chair: Mohamed Bennis, MA

S44.1 Mohamed Bennis Early postnatal alteration of the thalamocortical pathway leads to some schizophrenia-like symptoms
S44.2 Zakaria Ouhaz The implication of early thalamocortical cross-talk in the dynamic balance of excitation and inhibition in rat brain
S44.3 Didier Pinault Disruption of corticothalamic systems in a model of first-episode psychosis

S44.4 Anna Mitchell Thalamocortical Interactions important for cognitive functions: the role of the mediodorsal thalamus

S45 Room: Ballroom
THE BRAIN NETWORK OF LEVODOPA-INDUCED DYSKINESIA IN PARKINSON'S DISEASE
Chair: Salvatore Galati, CH

S45.1 Salvatore Galati Sleep-dependent plasticity in the emerging of levodopa-induced dyskinesias
S45.2 Alessandro Stefani *In vivo* electrophysiology of levodopa-induced dyskinesias
S45.3 Vincenza D'Angelo Dopamine-mediated metabolism of cAMP/cGMP and the levodopa-induced dyskinesias
S45.4 Alain Kaelin Opioidergic system as a potential target for preventing levodopa-induced dyskinesias?
S45.5 Giacomo Koch The role of cerebellum in levodopa-induced dyskinesias

S46 Room: Marie Louise 2
THE KEY ROLE OF CATECHOLAMINES IN STRESS, ADDICTION AND HUNTINGTON'S DISEASE
Chairs: Giulia Fois, FR and François Georges, FR

S46.1 Sebastian Fernandez Behavioural and cellular insights into nicotine and stress interplaycough
S46.2 Raffaella Tonini Functional remodeling of glutamatergic inputs to Locus Coeruleus neurons during the adolescence to adulthood transition
S46.3 Giulia Fois Context-switch promotes changes in the tonic and integrative electrophysiological properties of VTA dopaminergic neurons
S46.4 Christelle Glangetas Synaptic dysfunction in early symptomatic stage in a mouse model of Huntington's disease

S47 Room: Clermont Suite
PHYSIOPATHOLOGICAL MECHANISMS OF AIRWAY PROTECTION: COUGH AND SWALLOW REFLEXES
Chair: Christian Gestreau, FA

S47.1 Donald Bolser Role of the dorsal medulla in the neurogenesis of cough
S47.2 Ivan Poliacek Peripheral and central modulation of tracheobronchial cough
S47.3 Kofi-Kermit Horton New insights into swallow-breathing coordination
S47.4 Tara Bautista Brainstem-mediated control of protective upper airway reflexes and coordination with breathing
S47.5 Teresa Pitts Changes in cortical oropharyngeal sensory integration in Parkinson's disease

S48 Room: Marie Louise 1
BRIDGING THE GAP IN ADDICTION RESEARCH: CLINICAL STUDIES IN THE FIELD OF THE NOVEL PSYCHOACTIVE SUBSTANCES
Chair: Laura Orsolini, IT

S48.1 Laura Orsolini The 'endless trip': psychopathological and clinical issues related to the new synthetic hallucinogens
S48.2 Duccio Papanti From cannabis psychosis to spiceophrenia
S48.3 Levente Móró Harm reduction of novel psychoactive substances

S49 Room: Reading Room
ROLE OF UBIQUITIN PROTEASOME SYSTEM (UPS) IN SYNAPTIC PLASTICITY: IMPLICATIONS FOR NEW THERAPEUTIC
Chair: Patrizia Romualdi, IT

S49.1 Francesca Felicia Caputi Involvement of proteasome machinery in pain and addiction
S49.2 Diego Ruano Caballero Neuroinflammation, autophagy and proteasome
S49.3 Niki Chondrogianni Aging related diseases and ubiquitine proteasome

18.00–19.00
PLENARY LECTURE
Introducer: ***F. Eliseo Olucha Bordonau (ES)*** **Room: Ballroom**

The EBBS Plenary Lecture
NOTABLE AND SPECIFIC CATECHOLAMINERGIC INNERVATION IN THE PRIMATE THALAMUS. RELEVANCE IN PARKINSONISM
Carmen Cavada (ES)

20.00
Social Dinner

Thursday, 15th June 2017

07.30–12.00
Registration

08.30–09.00
Posters on display

09.00–10.00
PLENARY LECTURE
Introducer: ***Demian Battaglia (FR)*** **Room: Ballroom**

A DEVELOPMENTAL SCAFFOLD FOR HIPPOCAMPAL FUNCTIONSS
Rosa Cossart (FR)

10.00–10.30
Coffee break and Posters

10.30–12.15
Parallel Symposia

S50 **Room: Marie Louise 1**
BRAIN DIRECTED GENE TRANSFER STRATEGIES. WHAT WE LEARNED FROM PRECLINICAL RESEARCH FOR RARE DISEASES
Chairs: Giuseppe Ronzitti, FR and Fatima Bosch, ES

S50.1 Stefano Espinoza Dopamine transporter gene transfer in DAT-KO mice as a therapeutic strategy for DTDS
S50.2 Andrea Contestabile A neuro-specific gene therapy approach to treat cognitive impairment in Down syndrome by RNA interference
S50.3 Angela Gritti Preclinical modelling of lentiviral-mediated CNS gene transfer in rodents and non-human primates
S50.4 Giuseppe Ronzitti Rescue of the peripheral nervous system impairment in Pompe disease mouse model by liver gene transfer

S51 **Room: Ballroom**
THE MANY FACES OF T-TYPE CALCIUM CHANNELS
Chairs: Nathalie Leresche, FR and Regis Lambert, FR

S51.1 Nathalie Leresche T channel in synaptic transmission and plasticity
S51.2 Martin Heine Surface dynamics of T-type channels
S51.3 Emmanuel Bourinet Chasing the role of T-type channels in spinal pain circuitry

S52 **Room: Carlson Suite**
NOVEL APPROACHES TO BRAIN REPAIRS
Chairs: Azza Sellami, TUNIS and Bruno Frenguelli, UK

S52.1 Benkhalifa Rym Scorpion venom components as potential models for drug development in neuropathologies
S52.2 Olfa Masmoudi- Kouki Mechanisms involved in neuropeptides induced neuroprotection: Implications in oxidative stress neurodegeneration
S52.3 Bruno Frenguelli The purine salvage pathway as a therapeutic target in brain injury

S53 **Room: Carlson Suite**
ION CHANNELS AND DISEASES
Chair: Maria Cristina D'Adamo, MT

S53.1 Mauro Pessia Physiopathological role of CFTR channels
S53.2 Daniela Pietrobon Calcium channels and migraine
S53.3 Sonia Hasan Hypothyroidism with global developmental delay and seizure: role of mutations in K+ channels Kir4.1 (KCNJ10) and SLACK (KCNT1)
S53.4 Natascia Vedovato KATP channels and neonatal diabetes

S54 **Room: Marie Louise 2**
INTEGRATED REGULATION OF STRESS- AND AROUSAL-RELATED BEHAVIOURS BY RELAXIN-3/RXFP3 SYSTEMS
Chairs: Anna Blasiak, PL and Andrew Gundlach, AU

S54.1 João Covita Peptidergic modulation of pain: Effects of relaxin-3 on descending controls
S54.2 Anna Blasiak Cellular mechanisms associated with relaxin-3's orexigenic action and its interactions with arousal and stress systems
S54.3 Sherie Ma Role of GABA/peptidergic neurons in the nucleus incertus in control of arousal and emotional cognition
S54.4 Francisco E Olucha-Bordonau Modulation of septohippocampal function by nucleus incertus and relaxin-3/RXFP3 signalling

S55 **Room: Reading Room**
COMMON MECHANISMS IN PATHOPHYSIOLOGY OF NEURODEGENERATIVE DISORDERS
Chairs: Ioannis Sotiropoulos, PT and Sheela Vhas, FR

S55.1 Ioannis Sotiropoulos Novel synthetic microneurotrophins as neuroprotective and neurogenic agents against neurodegeneration
S55.2 Christina Dalla Stressful exposure and sex differences in brain vulnerability to depressive pathology: implications for dementia

S55.3 Ehud Cohen Cyclophilin malfunction underlies the development of distinct late-onset, neurodegenerative maladies
S55.4 Sheela Vhas Glucocorticoid signaling in neurodegenerative pathology of Parkinson's disease

12.30–14.00
Lunch
P15.1–P15.27 Poster Authors in Attendance

14.00–15.45
Parallel Symposia

S56 Room: Marie Louise 1
NEW PERSPECTIVES IN AMYOTROPHIC LATERAL SCLEROSIS: FROM MOLECULAR BASIS TO THERAPEUTIC INTERVENTION
Chair: Pignataro Giuseppe, IT

S56.1 Giuseppe Pignataro Preconditioning induced by low doses of LBMAA in SOD1G93A mice modulates the ionic transporter NCX3 leading to a state refractory to ALS
S56.2 Jonathan Cory Grima The nuclear pore complex is compromised in ALS
S56.3 Rachel Levy Targeting cytosolic phospholipase A2 in the spinal cord delay the development of ALS

S57 Room: Marie Louise 2
ION CHANNEL: A KEY FACTOR IN NEURONAL DYSFUNCTION
Chairs: Rami Yaka, IL, Alex Binshtok, IL and Avi Priel, IL

S57.1 Alexander M Binshtok The role of M-current in neuroinflammation
S57.2 Dario Di Francesco HCN-linked diseases in heart and brain
S57.3 Avi Priel Tightly regulated TRPV1 activation by Gq/GPCR
S57.4 Rami Yaka Exciting inhibitory control of VTA dopamine neurons by cocaine

S58 Room: Ballroom
BRAIN REPAIR AND REGENERATION
Chairs: Afsaneh Gaillard, FR and Fatiha Nothias, FR

S58.1 Monica Sousa The neuronal and actin commitment: why do axons need rings?
S58.2 Felipe Ortega Unravelling the mechanisms controlling neurogenic and oligodendrogliogenic lineages in the adult subependymal zone
S58.3 Gaillard Afsaneh Stem cell therapy for brain disorders
S58.4 Fatiha Nothias Regenerative biomaterial matrices for traumatic spinal cord repair in a rat experimental model
S58.5 Jorge Valero Gómez-Lobo Life factors shape adult neurogenesis and hippocampal dependent memory in animal models of Alzheimer's Disease

S59 Room: Clermont Suite
BRAIN IMAGING, HEALTHY AGING, SILENT AND APPARENT NEURODEGENERATIVE DISEASES
Chairs: Demian Battaglia, FR, Driss Boussaoud, FR and Saïd Boujraf, MA

S59.1 Boujraf Saïd Cognitive and emotional alteration in Parkinson's Disease, behavioral and cellular insights into nicotine and stress interplay
S59.2 Benzagmout Mohammed Cognitive and emotional alteration in Parkinson's Disease
S59.3 Driss Boussaoud Cognitive and emotional impairment in Type 2 Diabetes

S60 Room: Reading Room
STRESS RESPONDING IN MAMMALS: INTEGRATING CELLULAR SIGNALING, NEURONAL CIRCUITRY, AND BEHAVIOURAL OUTPUTS
Chairs: Youssef Anouar, FR and Lee E. Eiden, USA

S60.1 Nathalie Guerineau Stress-mediated remodeling of gap junctional communication in the adrenal medullary tissue
S60.2 Youssef Anouar Peptidergic control of oxidative stress in neuroendocrine cells
S60.3 Fatiha Chigr Food intake regulation in rat stress-induced anorexia
S60.4 Lee E. Eiden Neuropeptides in stress signaling from cells to circuits in the CNS

S61 Room: Carlson Suite
ASTROCYTIC CONTRIBUTIONS TO SYNAPTIC FUNCTIONS
Chairs: Stéphane Oliet, FR and Dimtri Rusakov, UK

S61.1 Christian Henneberger Rapid astrocyte morphology changes support epileptic activity
S61.2 Robert Zorec Astrocyte secretion capacity: fusion pore regulation
S61.3 Stephane Oliet Astrocytic IP3 receptors and their role in synaptic plasticity

S61.4 Dmitri Rusakov Deciphering the principles of astroglial signalling: experiments versus theory

15.45–16.15
Coffee break and Posters

Parallel Symposia

S62 Room: Ballroom
CAN ABSENCE SEIZURES BE PREVENTED?
Chair: Antoine Depaulis, FR

S62.1 Annika Lüttjohann Brain computer interfaces for absence seizure interruption and prevention
S62.2 Emilio Russo Mechanisms of antiepileptogenesis in absence models
S62.3 Nihan Carcak Exploring the initiation site of experimental absence seizures: novel pathways for patient-tailored treatment
S62.4 Cian McCafferty On the feasibility of seizure control and prevention in absence patients
S62.5 Guillaume Jarre Building up absence seizures: Network and cellular processes of absence epileptogenesis

S63 Room: Clermont Suite
FRAGILITY OF DECLARATIVE MEMORY: INSIGHT FROM DIFFERENT ANIMAL MODELS
Chair: Marighetto Aline, FR

S63.1 Gal Richter-Levin Juvenile stress and hippocampal function
S63.2 Azza Sellami Cellular bases of temporal binding assessed in trace fear conditioning in mice
S63.3 Aline Desmedt A deficit in declarative/contextual memory: basis of traumatic memory
S63.4 Nicole Etchamendy Defect in temporal binding is the source of aging-related decline in declarative memory

S64 Room: Clermont Suite
MONITORING BRAIN INFLAMMATION
Chairs: Galila Agam, IL and Sara Eyal, IL

S64.1 Sara Eyal Tracking inflammation in the epileptic rat brain by bi-functional fluorescent and magnetic nanoparticles
S64.2 Abed Azab Psychotropic drugs attenuate lipopolysaccharide-induced hypothermia by altering hypothalamic levels of inflammatory mediators
S64.3 Galila Agam Lithium as an anti-neuroinflammatory agent
S64.4 Hagit Eldar-Finkelman New modality of GSK-3 inhibition holds promise in treating neurodegenerative disorders

S65 Room: Marie Louise 1
FOOD INTAKE AND ENERGY METABOLISM CONTROL: PHYSIOLOGICAL AND PHYSIOPATHOLOGICAL ASPECTS
Chairs: Nicolas Chartrel, FR Mohamed Najimi, MA and Isabel Varela Nieto, ES

S65.1 Nicolas Chartrel 26RFa: a neuropeptide involved in the hypothalamic regulation of food intake and glucose homeostasis
S65.2 Carole Rovere- Jovene Nutritional lipids and brain inflammation: a new therapeutic pathway for the treatment of obesity?
S65.3 Mohamed Najimi Anorexia-induced stress involved in neurochemical and morphological changes
S65.4 Alexandre Benani Is the plasticity of brain feeding circuits involved in satiety?

S66 Room: Marie Louise 2
UNDERSTANDING AND PROTECTING PROGRESSIVE HEARING LOSS
Chair: Isabel Varela Nieto, ES

S66.1 Rami Saba Improving cochlear implant performance: from the bench to the bedside
S66.2 Philippe De Medina Synthetic oxysterols are novel hearing protective drugs
S66.3 Jiri Popelar Age-related alterations in the central auditory pathway
S66.4 Isabel Varela-Nieto Genetic reduction of insulin-like growth factor levels exacerbates otic damage via activation of JNK signaling and upregulation of pro-inflammatory cytokine production
S66.5 Francesca Cencetti Sphingosine 1 phosphate signaling modulates otic neurogenesis and neurodegeneration

S67 Room: Marie Louise 2
LANGUAGE LEARNING AND LEARNING DISORDERS
Chair: Mireille Besson, FR

S67.1 Mireille Besson Music training and word learning
S67.2 Johannes Ziegler Understanding developmental dyslexia: from causes to interventions
S67.3 Benjelloun Ghizlane Language and early interactions
S67.4 Smail Layes Developmental dyslexia in Arabic-speaking children: universal factors and orthographic features
S67.5 Khateb Asaid Word processing in Arabic and the diglossia question
S67.6 Driss Boussaoud Language learning: insight from the neurophysiology of social learning

18.00–19.00
PLENARY LECTURE
Introducer: *Y. Anouar (FR)* **Room: Ballroom**

AUTISM, SCHIZOPHRENIA AND ALZHEIMER' DISEASE: A COMMON THREAD FROM NEUROPEPTIDES TO BRAIN REGULATING GENES
Illana Gozes (IL)

19.00–20.00

Conclusion of MNS2017 **Room: Ballroom**

Best Poster PRIZES Ceremony
(1st ELSEVIER, 2nd XJENZA AND 3rd- THE RECEPTORS-SPRINGER/NATURE, Best Poster on Memory & Emotion Assc. V. De Castro)

Poster Session
12th June 2017

P12.1
ANTI-INFLAMMATORY DRUGS EXERT ANTIDEPRESSANT-LIKE EFFECTS AND REDUCE BRAIN LEVELS OF IL-6 IN RATS

Kaplanski J, Nassar A and Azab AN

P12.2
NEURONAL AND BEHAVIORAL CORRELATES OF PHASIC AND SUSTAINED FEAR IN FREELY BEHAVING MICE

Seidenbecher T, Remmes J, Daldrup, T, Lesting J and Pape H-C

P12.3
ALTERATIONS OF ULTRASONIC VOCALIZATION (USV) IN PURKINJE CELL SPECIFIC TSC1 KNOCKOUT MOUSE

Wiaderkiewicz J, Sługocka A, Głowacka M, Przybyła M, Nowacka-Chmielewska M, Chojnacka D and Barski JJ

P12.4
THE FAVORABLE IMPACT OF TIANEPTINE ON THE EVOKED BY PRENATAL STRESS DYSREGULATION OF CHEMOKINE-CHEMOKINE RECEPTOR AXIS IN BRAIN OF ADULT OFFSPRING RATS

Basta-Kaim A, Budziszewska B, Trojan E, Slusarczyk J, Chamera K, Głombik K and Kotarska K

P12.5
EFFECT OF CO-TREATMENT WITH ARIPIPRAZOLE AND ANTIDEPRESSANTS ON THE MK-801-INDICED CHANGES IN THE OBJECT RECOGNITION TEST IN RATS

Rogóż Z, G Skuza, Wąsik A and Lorenc-Koci E

P12.6
THE ANTIDEPRESSANT EFFECTS OF KETAMINE ON THE LATERAL HABENULA AND THE BEHAVIOUR OF NORMAL, RESTRAINT STRESS AND MATERNALLY DEPRIVED RATS

Crews-Rees A, Pierucci M, Delicata F, Benigno A and Di Giovanni G

P12.7
A NEUROIMAGING STUDY ABOUT EMOTIONAL PERSPECTIVE-TAKING; AN FMRI STUDY

Son JW

P12.8
BRAIN MORPHOLOGY AND FUNCTIONAL CHANGES ASSOCIATED WITH VISUAL SEXUAL AROUSAL IN MENOPAUSAL WOMEN

Kim G-W and Jeong G-W

P12.9
THE LONG TERM BRAIN EFFECTS OF BINGING ON ALCOHOL AND MARIJUANA IN ADOLESCENT TOBACCO USERS: A STUDY ON MOTIVATION IN OPERANT FOOD-REINFORCED RESPONDING. "THE PACEVILLE PROJECT: II"

Abela N, Pierucci M, Haywood K, Casarrubea M, Vella M, Crescimanno G, Benigno A and Di Giovanni G

P12.10
LORCASERIN, A SEROTONIN$_{2C/2A}$ RECEPTOR AGONIST, PREFERENTIAL MODULATES MESOLIMBIC VS. NIGROSTRIATAL DOPAMINERGIC FUNCTION: AN *IN VIVO* ELECTROPHYSIOLOGICAL AND MICRODIALYSIS STUDY

Ramos M, Pierucci M, Delicata F, Manem J, Benigno A, De Deurwaerdere P and Di Giovanni G

P12.11
TEMPORAL STRUCTURE OF THE MUSCULAR DYSTROPHY X-LINKED MOUSE BEHAVIOR TESTED IN OPEN FIELD

Faulisi F, Raso G, Morici G, Benigno A, Crescimanno G and Casarrubea M

P12.12
EPILEPSY AND AUTISM COMORBIDITY: ROLE OF GAIN-OF-FUNCTION DEFECTS OF Kir4.1 CHANNELS

Sicca F, Ambrosini E, Cozzolino O, D'Adamo MC, Marchese M, Minutolo F, Pessia M, Tuccinardi T, Valvo G, Ratto GM and Santorelli FM

P12.13
RECURRENT PATTERN DISCOVERY IN D1CT-7 MOUSE MODEL OF TIC-RELATED BEHAVIOR

Stockton E, Colangeli R, Di Maio R, Pierucci M, Benigno A, Brindisi M, Grillo A, Gemma S, Campiani G, Maccarrone M, Minetti P, Vella M, Butini S and Di Giovanni G

P12.14
RECURRENT PATTERN DISCOVERY IN D1CT-7 MOUSE MODEL OF TIC-RELATED BEHAVIOR

Santangelo A, Bortolato M, Di Giovanni G, Ricca V, Crescimanno G, Benigno A and Casarrubea M

P12.15
IMPACT OF BINGE-LIKE ETHANOL INTOXICATION DURING ADOLESCENCE ON VULNERABILITY TO ANXIETY AND DEPRESSIVE DISORDERS AT ADULTHOOD IN WISTAR RATS

Hicham El M, Tarik T, Abderrahim L, Ali O, Aboubaker E and Abelhalim M

P12.16
NEUROCHEMICAL AND BEHAVIOURAL EFFECTS OF COCAINE IN HATANO HIGH AND LOW-AVOIDANCE RATS

Piras G, Pisanu A, Lecca D, Serra GP, Valentini V and Di Chiara G

P12.17
PERINATAL EXPOSURE TO LEAD (PB) INDUCES ALTERATION IN GLYCOGEN METABOLISM IN DEVELOPING BRAIN OF RAT OFFSPRING

Baranowska-Bosiacka I, Gutowska I, Anna F, Łukomska A, Kolasa A, Pilutin A, Metryka E, Kupnicka P, Goschorska M and Chlubek D

P12.18
THE DIFFERENCE IN REACTIVENESS OF ALPHA1 ADRENERGIC RECEPTOR SUBTYPES AFTER TWENTY FOUR HOURS EXPOSURE TO ANTIDEPRESSANT DRUGS

Kusmierczyk J, Chmielarz P, Rafa-Zablocka K, Kowalska M and Nalepa I

P12.19
ADOLESCENCE VERSUS ADULTHOOD: DIFFERENCES IN BASAL DOPAMINE TRANSMISSION AND RESPONSE TO DRUGS OF ABUSE

Corongiu S, Dessì C and Cadoni C

P12.20
CHARACTERIZATION OF ENDOCANNABINOID SYSTEM IN AN ANIMAL MODEL OF BINGE EATING

Pucci M, Micioni Di Bonaventura MV, Bellia F, Falconi A, Maccarrone M, Cifani C and D'Addario C

P12.21
ANTIDEPRESSANT ACTION OF 2,5-DIMETHOXY-4-IODOAMPHETAMINE (DOI)

Kondev V

P12.22
THE LONG TERM BRAIN EFFECTS OF BINGING ON ALCOHOL AND MARIJUANA IN ADOLESCENT TOBACCO USERS: A BEHAVIOURAL AND NEUROCHEMICAL STUDY IN RATS: "THE PACEVILLE PROJECT I"

Haywood K, Casarrubea M, Abela N, Pierucci M, Camilleri SM, Crescimanno G, Benigno A, De Deurwaerdere P and Di Giovanni G

P12.23
SEROTONIN-2A AND -2C RECEPTOR MODULATION OF THE LATERAL HABENULA ACTIVITY IN THE CONTEXT OF NICOTINE ADDICTION

Delicata F

P12.24
ROLE OF THE KAPPA OPIOID SYSTEM ON THE FACILITATING EFFECTS OF PRENATAL ALCOHOL EXPOSURE ON LATER ALCOHOL INTAKE

Wille-Bille A, Pautassi RM and D'Addario C

P12.25
ENDOCANNABINOID STIMULATION IN PREGNANCY: WHAT HAPPENS TO THE OFFSPRING?

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Plenary Lectures

THE EMBO PLENARY LECTURE

IMMUNE PROTEINS IN BRAIN DEVELOPMENT AND SYNAPTIC PLASTICITY

Michela Matteoli
CNR Institute of Neuroscience and Humanitas Research Hospital Rozzano

In the last years evidence accumulated indicating that immune molecules influence brain development and physiology, also modifying the risk and/or severity of a variety of brain diseases. Although a number of immune molecules involved in inflammation have been found to regulate specific neuronal processes, the possibility that inflammatory cascades, either alone or in combination with a susceptible genetic background, may impact synapse formation and plasticity, thus leading to a disease condition, has not been addressed in a systematic way. The talk will report a series of evidence indicating that immune challenges, prenatally or postnatally delivered, impact synaptic protein networks, thus resulting in neuronal modifications typical of psychiatric diseases. Results from these studies will allow the identification of new targets suitable for innovative therapeutic intervention.

FENS SPECIAL LECTURE

KAINATE RECEPTORS IN MOOD DISORDERS

Juan Lerma
Instituto de Neurociencias, CSIC-UMH, San Juan de Alicante, Spain

Of the known ionotropic glutamate receptors, kainate receptors (KARs) are ubiquitous in the CNS and are present at both sides of the synapse. Pre- and postsynaptic KARs regulate both transmission of information and excitability in a synapse-specific manner. The involvement of KARs in synaptic plasticity is now clear and they play a fundamental role in epilepsy through the strategic control of network excitability. Proteins interacting with KARs subunits are being identified and functional studies have evidenced the existence of a dual signalling system: KARs may signal through ion flux or by activation of G-proteins. New data indicate their involvement in mood disorders. De novo copy number variation (deletion or duplication of a chromosomal region) of synaptic genes has been recently implicated as risk factors for mental retardation or autism. Amongst them is GRIK4, a gene coding for a high affinity KAR subunit, GluK4. The understanding of brain diseases requires the definition of the molecular, synaptic and cellular disruptions underpinning the behavioural features that define the disease. For this reason, we generated transgenic mice overexpressing grik4 in the forebrain. Surprisingly, GluK4 overexpression causes a significant increase in the efficiency of synaptic transmission in critical brain pathways, such as in the hippocampus and amygdala, in that amplitude of the AMPAR-mediated EPSCs as well as frequency and amplitude of mEPSCs were enhanced. These mice displayed social impairment, enhanced anxiety and depressive states. Together, these data indicate that a single gene variation in the glutamatergic system results in behavioural symptomatology consistent with autism spectrum disorders as well as in alterations in synaptic function in regions involved in social activity. This change in synaptic gain is a remarkable example on how altering information transfer through critical circuits may underlay mental disorders.

THE UNIVERSITY OF MALTA PLENARY LECTURE

MIRROR NEURONS: PAST, PRESENT, AND FUTURE

Giacomo Rizzolatti
Dipartimento di Neuroscienze, Università di Parma, Italy and Unità di Parma del CNR, Istituto di Neuroscienze and Department of Physiology and Biochemistry, University of Malta, Msida, Malta

An exciting discovery in neurosciences over the last years has been that of a mechanism that unifies action perception and action execution. The essence of this mechanism – the mirror mechanism - is the following. Each time individuals observe an action done by others,

a set of neurons that code that action in the motor system are activated. Because the observers are aware of the outcome of their motor acts, they also understand what the others are doing without the necessity of an intermediate cognitive mediation. In my talk, I will describe first the properties of the mirror mechanism in the monkey. I will present then evidence that humans also possess the mirror mechanism and that the anatomical location of parieto-frontal mirror networks of the monkeys and of humans closely coincide. I will show then the limits of the mirror mechanism in understanding others. I will stress that the mirror mechanism is, however, the only mechanism that allows a person to understand others' actions "from the inside" giving the observing individual a "first-person" grasp of other individuals' motor goals and intentions. I will conclude presenting new data that we recently obtained using intracortical (stereo-EEG) recordings in surgical patients with drug-resistant epilepsy. These data enabled us to generate highly resolved four-dimensional maps of human cortical activations. These maps indicate not only were the activations are located but also the timing of these activations. They show that an observed action is replicated in the observer's brain in visual and then in parietal and frontal areas. The end of the observed action is signaled by activation of area SII and of premotor cortex.

THE UNIVERSITY OF MALTA PLENARY LECTURE

UNIVERSITY OF MALTA
L-Università ta' Malta

CELLULAR AND NETWORK DYNAMICS OF EEG WAVES OF HEALTH AND DISEASE STATES

Vincenzo Crunelli
Neuroscience Division, Cardiff University, Cardiff, UK and Department of Physiology and Biochemistry, University of Malta, Msida, Malta

Cortico-thalamic networks are key to the generation of different EEG waves observed during various behavioural states, including the classical alpha waves of relaxed wakefulness, sleep spindles, delta waves and the slow (< 1 Hz) wave rhythm, which is the most significant EEG signature of non-REM sleep.

Though shaped by the neocortex, the EEG alpha rhythm is mainly driven by a subset of gap-junction-connected thalamocortical neurons that fire high-threshold bursts of action potentials, a novel intrinsic type of firing of these thalamic neuronal population, that is mediated by voltage-activated calcium channels. The firing of the other thalamocortical neurons during the alpha rhythm is finely tuned by local GABAergic interneurons, thus allowing information transfer from the periphery to the neocortex in output packets at alpha frequency. In contrast, sleep spindle and delta waves have been known for a long time to be fully and partly generated, respectively, by the thalamus and to be characterized by the intermitted and continuous presence, respectively, of low threshold, T-type calcium channel-mediated spikes (LTS).

The EEG expression of sleep slow (< 1 Hz) waves in naturally sleeping organisms requires the dynamic interaction of three cardinal neuronal oscillators: a predominantly synaptically-based cortical oscillator and two intrinsic, conditional thalamic oscillators. In particular, the isolated thalamus is capable of a synchronized network oscillation at < 1 Hz, and the absence of a thalamic output to neocortex results in slow waves at a lower frequency than under control conditions. Moreover, the prominent LTS that invariably initiates the UP states in thalamocortical neurons provide an ideal signal for initiating large-scale UP states in cortical networks. Importantly, the isolated cortex is endowed with powerful, though still not fully characterized, plasticity mechanisms that allows it to recover the natural slow (< 1 Hz) rhythm after few days of thalamic afferent disconnection

Thus, as alertness starts to decrease up to the deepest stages of non-REM sleep a characteristic feature of thalamic neuron firing dynamics is the expression of high-threshold bursts and LTSs. Since LTSs are generated by a global somato-dendritic electrical and biochemical signalling mechanism, I will argue that during these behavioural states LTSs not only represent a powerful tool to synchronize the firing dynamics of different thalamic and cortical neuronal populations but also provide the biochemical and electrical substrates for facilitating cellular and network thalamic and neocortical plasticity.

An abnormal cortico-thalamic network-dependent EEG rhythm is the 3 Hz spike-and-wave discharges (SWDs) that invariably accompanies the loss of consciousness during absence seizures (ASs). These seizures are a feature of many generalized genetic epilepsies and the defining seizure type of childhood/juvenile absence epilepsy. Despite being considered relatively benign, absence epilepsy involves learning difficulties, behavioural disorders and other psychiatric conditions, and monotherapy with gold-standard anti-absence drugs is only effective in only 50% of patients. I will discuss how advances in human genetics have provided limited breakthroughs, whereas imaging, electrophysiology and opto-

genetics (in humans and in different experimental models) have unravelled key pathophysiological mechanisms, thus providing novel potential therapeutic targets. In particular, it is now well documented that SWDs are not generalized form their start, but begin from a localized (often frontal) cortical area, from where they then spread to other cortical areas and to the thalamus. Moreover, a gain of function of thalamic extrasynaptic GABA-A receptors (which in genetic models is due to a decreased activity of the astrocytically located GABA transporter GAT-1) is both necessary and sufficient for the expression of absence seizures in all well-established rodent absence models. Moreover, knock-down of one of the extrasynaptic GABA-A receptor subunits rescues the experimental absence phenotype in both rat and mouse models. As a results of this enhanced thalamic inhibitory tone, thalamocortical neurons show a marked ictal decrease of their firing rate. This key experimental finding is supported by human data showing that in contrast to convulsive seizures anti-epileptic drugs that increase GABA-A receptor activity (tiagabin and vigabatrin) worsen human absence seizures and elicit them in healthy individuals. In my presentation, I will argue that there is no hyperexcitability in thalamocortical neurons during absence seizures and that drugs that are capable of directly or indirectly (via GAT-1) modulating the extrasynaptic GABA-A receptor function represent suitable alternatives to current medication for these genetic generalized epilepsies.

PLENARY LECTURE
THE ENDOCANNABINOIDS: LOOKING BACK AND AHEAD

Raphael Mechoulam (IL)
Hebrew University, Institute for Drug Research

Over the last few decades research on the cannabinoids has gone through several distinct phases:

A. Research on the plant cannabinoids [mostly on tetrahydrocannabinol (THC) and cannabidiol (CBD)];
B. Research on the endogenous cannabinoids [mostly on anandamide and 2-arachidonoyl glycerol (2-AG)];
C. Research on endogenous anandamide-like endogenous fatty acid amides with amino acids and ethanol amines.

Plant cannabinoids. While many dozens of plant cannabinoids are known today, most research is still on THC and CBD.

CBD was isolated in the late 1930's, but its structure was elucidated only in 1963. Pure THC was isolated in 1964, when its structure was elucidated. Later it was synthesized. The psychoactivity of cannabis preparations (marijuana, hashish etc) is mostly due to THC, but the other constituents may affect the activity of THC. Some of its metabolites are also psychoactive.

Thousands of publications have been published on the plant cannabinoids and some of them are already in use as therapeutic drugs. THC has been approved as a drug (named Marinol) for enhancement of appetite and is also used to prevent vomiting due to cancer chemotherapy.

Of particular interest is CBD, which does not cause the typical cannabis psychoactivity, but is a potent anti-epileptic drug and is used in many countries in paediatric epilepsy. It is being evaluated in other therapeutic areas (graft versus host disease, schizophrenia and autoimmune diseases for example).

The endogenous cannabinoids. Anandamide and 2-arachidonoyl glycerol (2-AG) were discovered in the 1990's. Both compounds bind to the cannabinoid receptors CB1 and CB2. They are involved in a very large number of human diseases, mostly as neuroprotective entities.

Endogenous fatty acid amides with amino acids and ethanol amines. A large number of compounds of these types have been discovered in brain and other tissues and some of them have been shown to be of major importance in a large spectrum of biological functions and diseases. Thus, oleoyl serine is an anti-osteoporotic molecule and arachidonoyl serine is a vasodilator and lowers brain damage.

Numerous pharmaceutical companies are now involved in research in all the above areas.

THE IBRO PLENARY LECTURE

NEURON-GLIA METABOLIC COUPLING: ROLE IN NEURONAL PLASTICITY AND NEUROPROTECTION

Pierre Magistretti
Brain Mind Institute, EPFL, Lausanne, Switzerland and Division of Biological and Environmental Sciences and Engineering, KAUST, Thuwal, KSA

A tight metabolic coupling between astrocytes and neurons is a key feature of brain energy metabolism. Over the years we have described two basic mechanisms of neurometabolic coupling. First the glycogenolytic effect of VIP - restricted to cortical columns - and of noradrenaline - spanning across functionally distinct cortical areas - indicating a regulation of brain homeostasis by neurotransmitters acting on astrocytes, as gly-

cogen is exclusively localized in these cells. Second, the glutamate-stimulated aerobic glycolysis in astrocytes. This metabolic response is mediated by the sodium-coupled reuptake of glutamate by astrocytes and the ensuing activation of the Na-K-ATPase. Both the VIP-and noradrenaline-induced glycogenolysis and the glutamate-stimulated aerobic glycolysis result in the release of lactate from astrocytes as an energy substrate for neurons.

We have recently revealed a second function of lactate, as a signaling molecule for plasticity. Indeed we have shown that lactate is necessary for long-term memory consolidation and for maintenance of LTP. The role of astrocyte-neuron lactate transfer is not restricted to the hippocampus nor to formation of aversive memories. Indeed in the basolateral amygdala lactate is necessary for the formation of an appetitive memory such as conditioned place preference for cocaine.

At the molecular level we have found that L-lactate stimulates the expression of synaptic plasticity-related genes such as Arc, Zif268 and BDNF through a mechanism involving NMDA receptor activity and its downstream signaling cascade Erk1/2. L-lactate potentiates NMDA receptor-mediated currents and the ensuing increases in intracellular calcium. These results reveal a novel action of L-lactate as a signaling molecule for neuronal plasticity.

THE EBBS PLENARY LECTURE

NOTABLE AND SPECIFIC CATECHOLAMINERGIC INNERVATION IN THE PRIMATE THALAMUS. RELEVANCE IN PARKINSONISM

Carmen Cavada
Dept. Anatomy, Histology and Neuroscience, Medical School, Universidad Autonoma de Madrid, Spain

We have studied the catecholaminergic innervation of the primate thalamus using immunohistochemistry for markers of the dopamine, noradrenaline and adrenaline phenotypes, i.e., dopamine and the dopamine transporter, dopamine-beta-hydroxylase and the noradrenaline transporter, and phenyletanolamine-N-methyltransferase, respectively.

The primate thalamus is innervated by dopamine, noradrenaline and adrenaline axons. The adrenaline axons are principally restricted to thalamic nuclei, or their portions, that are in the midline or close to the midline. The dopamine and noradrenaline innervations are remarkable in extent and density both in the macaque monkey and in the human thalamus. Moreover, the distributions of the dopamine and noradrenaline axons are highly diverse in the various primate thalamic nuclei. In general, the catecholaminergic innervation of the primate thalamus is denser and more widely distributed than that of the rodent thalamus. We have shown marked differences particularly for the dopamine innervation.

The noradrenaline innervation in the primate thalamus is, like the dopamine innervation, highly widespread and heterogeneous. It is most dense in midline and intralaminar nuclei. Within the intralaminar nuclei, the caudal part of the parafascicular nucleus, which projects to the limbic and associative striatum, holds very dense patches of noradrenaline axons. The mediodorsal nucleus, particularly its medial sector, is the most densely innervated within the association nuclei. Within the ventral nuclear group, subdivisions that are the targets of basal ganglia output nuclei receive a notable noradrenaline innervation. The first order sensory nuclei receive a weak-to-moderate density of noradrenaline axons; the dorsal lateral geniculate nucleus appears to be the less innervated throughout the thalamus. The distribution of noradrenaline axons in the primate thalamus suggests that they are mainly involved in modulating cortico-thalamo-cortical and cortico-thalamo-striatal circuits related to attention, cognition, motor, and limbic processing, and less so in primary sensory transmission.

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In consideration of the notable dopamine and noradrenaline innervations of thalamic regions included within cortico-basal ganglia-thalamic circuits, we are

examining them in parkinsonism using, in macaque monkeys, the neurotoxin 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) that destroys dopamine neurons expressing the dopamine transporter. MPTP has also a high affinity for the noradrenaline transporter. Biochemical data from other groups indicate that there is a marked noradrenaline loss in the thalamus of Parkinson's disease patients. In monkeys treated with MPTP, noradrenaline loss is notably more prominent than dopamine loss, and appears in monkeys with stable parkinsonism but not in early stages of the parkinsonian condition. By contrast to noradrenaline, dopamine levels are not consistently affected in the thalamus of parkinsonian monkeys. However, quantitative analysis of dopamine axons in the thalamus of MPTP treated monkeys shows a deafferentation that starts early in the parkinsonian condition, i.e., before motor symptoms appear. These findings indicate that specific changes in dopamine and noradrenaline metabolism and innervation are present in the parkinsonian thalamus; such changes need to be understood in detail as they may play a critical role in the pathogenesis of the condition.

PLENARY LECTURE
A DEVELOPMENTAL SCAFFOLD FOR HIPPOCAMPAL FUNCTIONS

Rosa Cossart
Institut National de la Santé et de la Recherche Médicale Unité 901, 13009 Marseille, France

Most adult cortical dynamics are dominated by a minority of highly active neurons distributed within a silent neuronal mass. If cortical spikes are sparse, spiking of single distinct neurons can impact on network dynamics and drive an animal's behavior. It is thus essential to understand whether this active and powerful minority is predetermined and if true to uncover the rules by which it is set during development. In this talk, I will present data supporting the possibility that birthdate is a critical determinant of neuronal network function into adulthood. More specifically, we reason that neurons that are born the earliest are primed to participate into adult network dynamics. This hypothesis is considerably fed by our past work aiming at understanding how cortical networks function and assemble during development. Hence, we have shown that an early birthdate: (1) specifies the specialization of GABA neurons with a hub function, that orchestrate perinatal network dynamics in the mouse hippocampus and develop into long-range projecting GABA neurons into adulthood; (2) delineates a subtype of CA3 glutamate neuron with a "pacemaker" function in the absence of fast GABAergic transmission. I will first briefly present this set of published data.

To test the hypothesis that early born cells are primed to be recruited in the active minority of neurons in the adult hippocampus, we needed to probe microcircuit function *in vivo*, where the extensive and long-range connectivity of these cells is preserved. I will show how we have translated from the *in vitro* to the *in vivo* situation, our multidisciplinary method to investigate structure-dynamics relationship in cortical networks. Using this approach, I will present data showing the recruitment of different developmental and morpho-functional subtypes of CA1 neurons into assemblies spontaneously bound together into chained sequences of neuronal activation when the hippocampus is in an "internal" mode of operation i.e. during run under the sole influence of self-motion cues or during immobility when sharp-wave ripple-associated events occur. We will compare the functional organization thus obtained to the one shaped by external environmental factors.

PLENARY LECTURE
AUTISM, SCHIZOPHRENIA AND ALZHEIMER' DISEASE: A COMMON THREAD FROM NEUROPEPTIDES TO BRAIN REGULATING GENES

Illana Gozes
The Lily and Avraham Gildor Chair for the Investigation of Growth Factors, Director, Elton Laboratory for Neuroendocrinology, Department of Human Molecular Genetics and Biochemistry, Sackler Faculty of Medicine, Adams Super Center for Brain Studies and Sagol School for Neuroscience, Tel Aviv University, Israel

Our original cloning of the gene coding for vasoactive intestinal peptide (VIP), led to the identification of VIP's involvement in synapse formation and neuroprotection through our discoveries of activity-dependent neurotrophic factor (ADNF) and activity-dependent neuroprotective protein (ADNP). To precisely delineate VIP and ADNP activities in the whole animal, we established transgenic animals, showing that manipulating VIP content impacts cognition in the mouse. As for mouse Adnp, complete knockout results in severe neuronal tube closure defects and embryonic death at the time of neural tube closure. Adnp haploinsufficient mice survive and show cognitive and social deficiencies, with pathologies resembling autism and Alzheimer's disease. Delineating the mechanism of action of ADNP we discovered binding to the SWI/SNF chromatin remodeling complex and heterochromatin 1 alpha, and direct interaction with specific gene promoters (e.g. the major risk gene for Alzheimer's disease, apolipoprotein E). We have further discovered interactions with proteins associated with RNA splicing as well as with proteins regulating translation, like eukaryotic initiation factor 4E (Eif4e). In the cell cytoplasm, ADNP further interacts with the autophagy mechanism, binding to microtubule associated protein 1 light chain 3 (LC3) and to micro-

tubule end binding proteins (EBs). These multiple interactions, with key regulatory proteins, was further associated with the fact that Adnp regulates > 400 genes during embryonic development and thousands of genes postnatally, with age and sex differences. Importantly, ADNP was recently identified as one of the major genes mutated de novo, leading to autism. Furthermore, blood borne ADNP levels correlate with IQ tests in elderly individuals. To try and combat ADNP deficiencies, we have designed and synthesized an ADNP – derived peptide, drug candidate, NAP (NAPVSIPQ), also known as davunetide, CP201. Containing the EB1,3 interacting domain SIP, NAP directly interacts with microtubules to induce the formation of dendritic spines and brain synaptic plasticity. While enhancing ADNP interaction with microtubules as well as the autophagosome, NAP provided for enhanced microtubule dynamics and active autophagy. In animals, NAP provided protection against neuronal toxicities and genetic manipulations associated with autism, schizophrenia and Alzheimer's disease. Based on the NAP binding site, a novel drug candidate was developed, namely SKIP, enhancing axonal transport and protecting cognition. NAP has shown clinical efficacy and together with SKIP, both compounds are poised to further clinical development (http://www.coronisns.com/).

Symposia

S1 Room: Ballroom
NEW DIRECTIONS IN SEROTONIN RECEPTOR RESEARCH AND THERAPEUTICS

Chair: Umberto Spampinato, FR

S1.1
AN IGNORED NEURAL RECEPTOR: THE 5-HT$_{2B}$ RECEPTOR STRATEGIES FOR THERAPEUTICS IN NEUROPSYCHIATRIC DISORDERS

Umberto Spampinato
Bordeaux University, Bordeaux, France; Inserm U1215, Neurocentre Magendie, Physiopathology of Addiction Group, Bordeaux, France

The central serotonin$_{2B}$ receptor (5-HT$_{2B}$R) is a well-established modulator of dopamine (DA) neuron function in the mammalian brain. Indeed, recent studies have shown that 5-HT$_{2B}$R antagonists exert a differential control on ascending DA pathways, resulting in increased, decreased and unaltered DA release in the medial prefrontal cortex (mPFC), the nucleus Accumbens (NAc) and the striatum, respectively. In keeping with the role of DA pathways in the symptomatology of schizophrenia, these findings led to the suggestion that 5-HT$_{2B}$R antagonists could be an interesting pharmacological tool for treating schizophrenia.

However, the mechanisms underlying the opposite effect of 5-HT$_{2B}$R antagonists on mPFC and NAc DA release remain unknown to date. The central 5-HT$_{1A}$R, a key pharmacological target in the therapeutic benefit of atypical antipsychotic drugs, could participate in this interaction, as 5-HT$_{1A}$R agonists are known to increase mPFC DA outflow and decrease it in the NAc, these effects likely resulting from the stimulation of mPFC 5-HT$_{1A}$Rs.

The present *in vivo* microdialysis study aimed at assessing this hypothesis, by using two selective 5-HT$_{2B}$R and 5-HT$_{1A}$R antagonists, RS 127445 (0.16 mg/kg, i.p.) and WAY 100635 (0.16 mg/kg, s.c.), respectively. First, the peripheral and intra-dorsal raphe nucleus (DRN, 0.032 µg/0.2 µl) injection of RS 127445 increased 5-HT release in the mPFC. Second, RS 127445-induced changes of DA release in the mPFC and the NAc were blocked by the peripheral or the intra-mPFC (0.1 µM) WAY 100635 administration.

These results demonstrate the existence of a functional interplay between mPFC 5-HT$_{1A}$Rs and DRN 5-HT$_{2B}$Rs in the control of DA mesocorticolimbic system, and highlight the clinical interest of this interaction as both receptors represent an important pharmacological target for the treatment of schizophrenia.

S1.2
SEROTONIN$_3$ RECEPTOR BLOCKADE POTENTIATES THE ANTIDEPRESSANT RESPONSE

Nasser Haddjeri
Stem Cell & Brain Institute, INSERM U846, Université de Lyon, F-69373, France

The therapeutic effect of current antidepressant drugs appears after several weeks and a significant number of patients do not respond to treatments. Hence, more effective strategies are urgently needed. Hence, we recently examined whether the pharmacological blockade of serotonin 3 receptors [5-hydroxytryptamine (5-HT$_3$) receptors] potentiates the antidepressant response of selective serotonin reuptake inhibitors (SSRIs), the most prescribed class of antidepressants.

The effects of acute administrations of the selective 5-HT$_3$ receptor antagonist ondansetron and of the SSRI paroxetine, alone and in combination, were evaluated with respect to 5-HT neuronal firing rate in the dorsal raphe nucleus (DRN) of anesthetized rats and on extracellular levels of 5-HT in the ventral hippocampus of freely moving rats. The results showed that ondansetron partially prevented the suppressant effect of paroxetine on DRN 5-HT neuronal firing and enhanced the paroxetine-induced increase of hippocampal extracellular 5-HT release. Accordingly, acute dosing of the multimodal antidepressant vortioxetine inhibited DRN 5-HT neuronal firing activity more potently than the SSRI fluoxetine and the 5-HT$_3$ receptor partial agonist SR57227 prevented the suppressant effect of vortioxetine, but not of fluoxetine.

These results indicated that the 5-HT$_3$ receptor participates in both mood regulation and the antidepressant effect of SSRIs, and that 5-HT$_3$ receptor antagonism contributes to the peculiar antidepressant property of vortioxetine.

S1.3
5-HT$_{2C}$ RECEPTORS AS A THERAPEUTIC STRATEGY IN SUBSTANCE USE DISORDERS: FOCUS ON COCAINE

Malgorzata Filip
Institute of Pharmacology, Polish Academy of Sciences, Laboratory of Drug Addiction Pharmacology, Smętna Street 12, PL 31-343 Krakow, Poland

There is a strong evidence that the brain serotonin$_{2C}$ receptors (5-HT$_{2C}$Rs) exert the inhibitory control over goal-directed behaviors. Animal data show that 5-HT$_{2C}$R activation efficiently alters drug and food taking and seeking behaviors, while clinical findings indicate

that the novel selective agonist at 5-HT_{2C}Rs lorcaserin is safe and well-tolerated in the treatment for chronic weight management in obese patients. 5-HT_{2C}R are also important brain target for mood disorders, including depression. As depression and substance (e.g., cocaine) use disorders are common concurrent diagnoses, in this study bilateral olfactory bulbectomy (an animal model of depression) with a variety of self-administration and extinction/reinstatement procedures were employed to study the influence of selective 5-HT_{2C}R agonists (Ro 60-0175 and WAY 161503) on cocaine reinforcement and reinstatement. Ro 60-0175 (3–10 mg/kg), but not WAY 161503 (1–3 mg/kg) significantly reduced cocaine self-administration in sham and bulbectomized animals. Ro-60175 in doses 0.3–3.0 mg/kg potently decreased the cocaine–primed reinstatement of cocaine seeking in both phenotypes. WAY 161503 (0.3–3.0 mg/kg) effectively protected against cocaine-induced relapse in rats with depressive phenotype; the same inhibition was observed for the drug highest dose (3 mg/kg) in sham operated controls. Both 5-HT_{2C}R agonists (0.1–1.0 mg/kg) blocked the drug-associated cue-evoked reinstatement behavior. Altogether, these preclinical findings show that 5-HT_{2C}R agonists reduce cocaine-seeking in normal and depressive-like behavioral phenotypes.

S1.4
NOVEL SEROTONINERGIC TARGETS AND STRATEGIES IN THE TREATMENT OF MAJOR DEPRESSIVE DISORDER

Francesco Artigas

Department of Neurochemistry and Neuropharmacology, IIBB-(CSIC-IDIBAPS- CIBERSAM), 08036 Barcelona, Spain

Major depressive disorder (MDD) is a severe psychiatric syndrome with high prevalence and socioeconomic impact. Current antidepressant treatments are based on the blockade of serotonin (5-hydroxytryptamine, 5-HT) and/or noradrenaline transporters. These drugs show slow onset of clinical action and limited efficacy, partly due to the activation of physiological negative feed-back mechanisms operating through autoreceptors (5-HT_{1A}, 5-HT_{1B}, α2-adrenoceptors) and postsynaptic receptors (e.g., 5-HT_3). As a result, acute treatments with clinically-relevant doses of reuptake inhibitors increase extracellular (active) 5-HT concentrations in the midbrain raphe nuclei but not in forebrain. The prevention of these self-inhibitory mechanisms by antagonists of the above receptors augments preclinical and clinical antidepressant effects. Hence, the mixed β-adrenoceptor/5-HT_{1A} partial agonist (r antagonist) pindolol accelerated, and in some cases enhanced, the clinical action of selective serotonin reuptake inhibitors (SSRI). This strategy has been incorporated into two new multi-target antidepressant drugs, vilazodone and vortioxetine, which combine 5-HT reuptake inhibition and partial agonism at 5-HT_{1A} receptors. Vortioxetine shows also high affinity for other 5-HT receptors, particularly ionotropic 5-HT_3 receptors, located in cortical and hippocampal GABA interneurons, whose activation markedly increases interneurons activity. Blockade of 5-HT_3 receptors by vortioxetine increases pyramidal neuron activity in prefrontal cortex (PFC) and evokes a greater increase of 5-HT release than classical antidepressants, two effects mediated by $GABA_A$ and $GABA_B$ receptors. Interestingly, subchronic vortioxetine administration at clinically-relevant oral doses also increases pyramidal neuron discharge in PFC. These effects may be involved in the pro-cognitive preclinical and clinical action of vortioxetine.

In recent years, we have also been examining the potential usefulness of RNAi strategies to evoke antidepressant-like effects in rodents. Our strategy has been to develop conjugate small interfering RNA (siRNA) molecules in which the siRNA sequence was covalently bound to the SSRI sertraline (conjugated siRNA; C-siRNA) in order to selectively accumulate it by the dense network of 5-HT axon terminals in brain. Low amounts (typically 0.5–2.0 nmol/day) of C-siRNA directed against 5-HT_{1A} receptors (5-HT_{1A}-R) and the serotonin transporter (SERT) were then administered intranasally to mice and C-siRNA sequences were localized in raphe serotonin neurons. Using this strategy, we discerned the role of pre- and postsynaptic 5-HT_{1A}-R in the response to stress, the anxiety phenotype and the response to antidepressant treatments. Interestingly, the selective silencing of presynaptic 5-HT_{1A} autoreceptors was sufficient to elicit antidepressant-like effects in mice thanks to the increased capability of serotonergic neurons to release serotonin during stressful situations.

Likewise, we employed this approach to silence SERT expression/function. Intranasal administration of a C-siRNA targeting SERT (C-SERT-siRNA) evoked rapid and robust antidepressant-like responses in mice, including elevated forebrain 5-HT levels, presynaptic 5-HT_{1A}-R desensitization, increased hippocampal neurogenesis and expression of trophic factors, and increased dendritic complexity. Further, C-SERT-siRNA reversed depressive-like behaviors in a mouse model of depression. C-SERT-siRNA evoked all these responses in 1 week whereas SSRI fluoxetine required 1 month. In addition, we are using this strategy to knockdown other genes potentially involved in stress resilience (e.g., TASK-3 channels; Ferrés-Coy et al., manuscript in preparation) and to target catecholamine neurons, by linking siRNA or antisense oligonucleotide sequences to the respective transporter inhibitors. Although it is still soon to know the impact of RNAi-based therapies on

MDD treatment, our approach to deliver C-siRNA sequences to serotonin neurons through the intranasal route has proven successful in order to elicit rapid and robust antidepressant-like actions in rodents, showing a high potential translational value.

S2 Room: Clermont Suite STIMULATING THE TOURETTE BRAIN – OBTAINING THERAPEUTIC RELIEF AND MECHANISTIC INSIGHT

Chairs: Christine Winter, DE and Laura Pozzi, SE

S2.1 BRAIN STIMULATION TECHNIQUES IN THE CONTEXT OF REPETITIVE DISORDERS

Christine Winter
Department of Psychiatry and Psychotherapy, Medical Faculty Carl Gustav Carus, Technische Universität Dresden, Germany

Tourette Syndrome (TS) is one of the most enigmatic neuropsychiatric disorders. Core symptoms comprise involuntary repetitive movements worsening under stress and upon amphetamine challenge. In its severe form TS constitute a greatly defacing and highly stigmatizing condition. Still, it is underrepresented in preclinical studies with only very few animal models that would allow insights into its pathophysiology and consequently contribute to the development of novel treatments.

Interdisciplinary evidence suggests a pivotal role of the dopamine system and the corticostriatal circuitry in TS pathology. Reduced tonic extracellular, increased presynaptic, and pharmacologically released intrasynaptic dopamine contents as well as increased dopamine receptor availability, suggests an overactive dopamine transporter (DAT) as one important mechanism underlying TS pathophysiology. Genetic association studies support this notion by selectively demonstrating alterations in DAT genes associated with TS and tic severity. On this basis we created a transgenic rat model that via pronuclear microinjection overexpresses the DAT gene. Neurobiological and behavioral studies were conducted on adult male hemizygous DAT-transgenic rats (DAT-tg) ubiquitously overexpressing DAT in the corticostriatal and associated networks.

DAT-tg rats showed increased grooming upon stress exposure and upon d-amphetamine administration at a dosage ineffective in wt rats. This low-dose amphetamine induced repetitive behavior suggests a susceptibility to amphetamine also found in TS patients. It manifests over time with a maximal expression 80–120 min after drug administration. Testing Tourette-pharmacotherapy, we found clonidine to specifically reduce repetitive behavior in DAT-tg rats. Further strengthening that the observed repetitive behavior relates especially to TS symptomatology, the serotonin reuptake inhibitor fluoxetine known to ameliorate repetitive symptoms in obsessive compulsive disorders but not in TS, did not selectively affect repetitive behavior in DAT-tg rats. Neurobiologically, we found that DAT-tg rats show relative overexpression of DRD1+DRD2 and decreased tissue dopamine levels in the orbitofrontal cortex, the caudate putamen and the nucleus accumbens. This was further paralleled by increased striatal MAO enzymatic activity, previously linked to TS. Increased MAO activity leads to increased dopamine turnover, resulting in decreased in dopamine levels, both found in DAT-tg rats. Further, DAT-tg displayed region-specific increments and decrements in GABAergic and glutamatergic contents in the corticostriatal circuit as well as a reduction of PV+ interneurons in DAT-tg rats as compared to wt rats. In line with clinical data, this reduction was restricted to the caudate putamen. On a brain volumetric level, DAT-tg rats displayed increased hippocampal volumes alongside normal whole brain and striatal volumes. Increased hippocampal volumes have been suggested to constitute a compensatory response in TS.

Together, our findings support the hypothesis that the DAT may constitute one important key component in TS pathophysiology and that DAT overexpression might be of relevance for further comprehension of neurobiological mechanisms underlying especially TS.

S2.2 BRAIN STIMULATION TECHNIQUES IN THE CONTEXT OF REPETITIVE DISORDERS

Henriette Edemann-Callesen
Klinik für Psychiatrie und Psychotherapie, Universitätsklinik Carl Gustav Carus – TU Dresden; International Graduate Program Medical Neurosciences, Charité – Universitätsmedizin Berlin, Germany

Treating Tourette Syndrome (TS) remains a challenge. To improve treatment options, focal brain stimulation techniques have been suggested as a mean to specifically target and modulate pathology-relevant brain areas. These techniques include the non-invasive transcranial direct current stimulation (tDCS) and the invasive deep brain stimulation (DBS) approach. Until now there is clinically no agreement as to which target and stimulation parameters constitute the most optimal. Thus, to move forward with the establishment of these therapies for TS, there is the need for a thorough examination into the efficacy of tDCS and DBS on repetitive symptomatology. This remains a challenge in the clinic

yet can be overcome preclincally.

We assessed the effect of tDCS and DBS in the dopamine transporter overexpressing (DAT) rat. DAT rats have shown to exhibit time-locked induction of repetitive behaviour following amphetamine injection. In this study, rats were challenged in the repetitive behavioural paradigm. Stimulation was applied immediately after amphetamine injection and behaviour was scored during the time of repetitive behaviour manifestation. DBS was applied at either low (10 Hz) or high (130 Hz) frequency to cortical (orbitofrontal cortex, medial prefrontal cortex and motor cortex) and subcortical regions (caudate putamen, enteropeduncle and centromedial parafasicularis). Anodal and cathodal tDCS was applied over the frontal cortex at current intensities either at 100 µA, 200 µA and 300 µA.

Results show that anodal tDCS (200 µA) applied over the frontal cortex significantly reduces the occurrence of repetitive behaviour in the DAT rats. In addition, high frequency DBS decreases repetitive behaviour when applied to the motor cortex, the caudate putamen and the enteropenduncle. No other stimulation type or intensity yielded a therapeutic effect.

Results show that especially modulation of the motor cortex seems to be involved in the therapeutic effect of these stimulation techniques. This indicates that stimulation of the motor loop within the basal-ganglia-thalamocortical circuit is involved in the manifestation of repetitive behavior. As such, targeted stimulation of pathology-relevant brain areas can indeed yield therapeutic relief in the context of repetitive behavior. This thorough investigation sets the stage for further investigation into the usage of these types of stimulation techniques as a treatment for TS.

S2.3
EMPLOYING OPTOGENETICS TO ELUCIDATE THE FUNCTION OF PARVALBUMIN (PV+) INTERNEURONS IN THE CONTEXT OF TOURETTE SYNDROME

Laura Pozzi
Department of Neuroscience, Karolinska Institutet, Stockholm, Sweden

Repetitive behaviors are found in a number of neuropsychiatric disorders. Tourette syndrome, once considered rare, is now known to affect approximately 1% of the population worldwide. The underlying neuropathology remains inadequately understood and treatment for severe intractable cases remains challenging. Converging evidence implicates a marked disruption in the distribution of GABAergic parvalbumin (PV+)-positive cells within the basal ganglia of patients affected by Tourette syndrome. These interneuron disturbances are reported to be specific to sensorimotor regions of the striatum paralleling changes reported in the sensori-motor cortex. In particular, the aberrant activity of PV+ expressing interneurons could underlie the behavioral manifestation of Tourette syndrome.

Our work aims to characterize the precise contribution of striatal parvalbumin-expressing GABAergic interneurons to the pathophysiology of repetitive behaviors and their subsequent suppression. To tackle this question we target striatal PV+ interneurons in naïve rats or in rats displaying repetitive behaviors, to provide causal evidence for their role in the development of Tourette's-like behavior. Our specific goals are: (1) to apply in vivo optogenetics to activate and to reduce the activity of striatal PV+ interneurons in transgenic PV-Cre rats, (2) to combine optogenetic manipulation of striatal PV+ interneurons and calcium imaging in transgenic PV-Cre rats with apomorphine, thus mimicking the pathological states hypothesized to be relevant in Tourette syndrome and, (3) to determine the behavioral consequences of targeted PV+ interneuron control in naïve and apomorphine-treated animals.

Overall, we want to evaluate if control of PV+ interneurons could provide for a novel type of intervention therapy to counterbalance the aberrant circuit activity in Tourette syndrome.

S2.4
ADAPTIVE DEEP BRAIN STIMULATION (aDBS) IN TOURETTE SYNDROME

Alberto Priori
III Department of Health Sciences, University of Milan & Ospedale San Paolo, Milan, Italy. Clinical Center for Neurostimulation, Neurotechnology and Movement Disorders, Fondazione IRCCS Ca' Granda, Ospedale Maggiore Policlinico, Milan, Italy

Deep brain stimulation (DBS) has emerged as a rising therapy for the treatment of neuropsychiatric disorders as Tourette Syndrome (TS). The DBS targets mainly used to date in TS are located within the basal ganglia-thalamo-cortical circuit compromised in this syndrome: the ventralis oralis/centromedian-parafascicular (Vo/CM-Pf) nucleus of the thalamus and the nucleus accumbens (NA). Current DBS treatment deliver electrical stimulation continuously, and are not designed to adapt to patients' symptoms contributing to unwanted side effects. Moreover, continuous DBS can lead to rapid battery depletion, which necessitates frequent battery replacement surgeries.

The adaptive deep brain stimulation (aDBS) controlled by neurophysiological biomarkers is considered as one of the most promising approaches to optimize clinical benefits and to limit side effects of DBS. The aDBS consists of a closed-loop system designed to meas-

ure and analyse a control variable reflecting the patient's clinical condition and to modify on-line stimulation settings to improve the treatment. LFPs, a sum of pre and post synaptic activities arising from large neuronal populations, directly recorded from electrodes implanted for DBS, theoretically can represent a reliable correlate of patient's clinical status in patients with TS. The well-established LFP-clinical correlations in patients with Parkinson's Disease provided in the last years the rationale for developing and implementing new aDBS devices whose efficacy was successfully tested in humans.

Conversely only few studies investigated the LFP activity recorded from DBS target structures and its relation to clinical symptoms in TS.

To do so, we recently acquired and analyzed, in patients with TS after DBS electrode implantation, LFPs at rest, during voluntary and involuntary movements (tics) and during ongoing DBS.

We enrolled patients with severe TS, satisfying DSM-IV-TR, refractory to standard drug treatment: patients with motor tics were implanted in Vo/CM-Pf nucleus and patients with motor tics and severe psychiatric comorbidities in the NA. The recording sessions included: (1) LFP at rest; (2) LFP for the time required to observe at least 15 tics; (3) LFP during 15 voluntary movements; (4) LFP during ongoing DBS. We calculated LFP power spectral density for different frequency bands: low-frequency (LF), alpha and beta bands.

VO/CM-Pf LFPs showed two main oscillatory activities, one in the LF band (2–7 Hz) and one in the alpha band (8–13 Hz) whereas AN LFPs only one main oscillation in the LF band. The most consistent Vo/CM-Pf power modulations during voluntary movements were in the LF and in the alpha bands: power increases from baseline started before movement onset. During tics LF and alpha power increased from baseline. Moreover, DBS induced specific LFP changes: LF power increased whereas the alpha band decreased from baseline during ongoing DBS and returned to baseline power after DBS was turned off.

TS patients have an oscillatory pattern specifically related to the recording brain structure and consequently to clinical manifestations. Vo/CM-Pf nucleus of thalamus is involved in the movement execution and in the pathophysiology of TS. Moreover, the oscillatory patterns in TS were specifically modulated by DBS treatment according to the improvement of TS symptoms. These preliminary findings suggest that LFPs recorded from DBS target can be used to control new aDBS device capable of closed-loop stimulation responsive to the symptoms of TS.

S3 Room: Marie Louise 1
PRECLINICAL AND TRANSLATIONAL APPROACHES FOR THE INVESTIGATION OF DRUG ADDICTION

Chairs: Marcello Solinas, FR and Roberto Ciccocioppo, IT

S3.1
PERSISTENT NEUROADAPTATIONS FOLLOWING EXTENSIVE COCAINE SELF-ADMINISTRATION AND STRATEGIES TO BOOST RECOVERY PROCESSES

Marcello Solinas
"INSERM Team Neurobiology and Neuropharmacology of Addiction" Laboratory of Experimental and Clinical Neurosciences INSERM U-1084 University of Poitiers, France

Modern theories of addiction postulate that excessive use of drugs induces neuroadaptations in brain areas such as the frontal cortex, the mesolimbic dopamine system, the extended amygdala and the striatum that are involved in several processes encompassing motivation, salience attribution, stress and cognition. These neuroadaptations would persist, at least in part, after discontinuation of drug use and would render individuals vulnerable to life events and situations that trigger craving and incapable to resist relapse. We will present results obtained in our laboratory that identify transient and persistent changes in brain activity that are associated with escalation of cocaine self-administration. We will also discuss about strategies that could facilitate recovery processes and decrease the risks of relapse.

S3.2
SURGICAL STRATEGY FOR ADDICTION BASED ON RATS AND MONKEYS STUDIES USING DEEP BRAIN STIMULATION APPLIED AT THE LEVEL OF THE SUBTHALAMIC NUCLEUS

Christelle Baunez
Institut de Neurosciences de la Timone (INT), UMR7289, CNRS & Aix-Marseille Université, Marseille, France

Surgical strategy to treat addiction has been suggested in the last few years after the success of deep brain stimulation (DBS) for the treatment of psychiatric disorders such as depression and obsessive compulsive disorders. The choice of the target for such treatment is

a critical step and is under debate for addiction. The target tested so far in human has been mostly the nucleus accumbens with little success. Based on our studies in the rats, we have proposed the subthalamic nucleus (STN) as a promising target. We have indeed previously shown that lesion or DBS of the STN could reduce motivation for cocaine while increasing motivation for sweet food reward. Since reducing the motivation to take the drug without reducing all forms of motivation is the challenge to treat cocaine addiction, STN DBS could be the interesting strategy. We have further tested this hypothesis by testing the effects of STN DBS on models of addiction criteria using the escalation of cocaine intake, the resistance to punishment in rats. In order to validate the translation to primates, with have tested the effects of STN DBS on motivation for cocaine and apple sauce in monkeys. All these results will be reviewed. They strongly support STN DBS as a possible strategy for the treatment of addiction to cocaine and possibly other substances of abuse.

S3.3
TRANSLATIONAL STRATEGIES TO DEVELOP NEW MEDICATIONS FOR ADDICTION

Roberto Ciccocioppo
University of Camerino, School of Pharmacy, Pharmacology Unit, Camerino, Italy

Addiction is an etiologically and clinically heterogeneous disorder in which uncontrollable urge to use the drug represent a core symptom. Exposure to psychoactive agents is a necessary precondition. However, environmental and heritability factors can play a dramatic role in controlling individual vulnerability to developing addiction. The complexity of drug addiction severely hampers the development of novel chemical entities to treat this psychiatric condition that remains a largely unmet medical need. A greater understanding of the mechanisms leading to drug abuse in addition to characterization of genetic factors responsible for shaping specific biological vulnerability traits, can help to deconstruct this complex disorder, and possibly to develop more efficacious treatments. To this end, it is critical to develop a translational framework that links alterations at the molecular level, to changes in neuronal function, and ultimately to changes at the behavioral and clinical levels. Translational phenotypes can be identified by the combination of animal and human studies designed to elucidate the neurofunctional, neuroanatomical, and pharmacological mechanisms underlying the etiology of drug addiction. Here we will discuss critical aspect of translational research offering examples of promising targets for medication development. Work supported by grant (NIAAA: RO1 AA017447, and RO1 AA014351)

S3.4
ROLE OF THE ENDOCANNABINOID SYSTEM IN FOOD-INDUCED ADDICTIVE-LIKE BEHAVIOR

Rafael Maldonado
Laboratorio de Neurofarmacología. Universitat Pompeu Fabra. Parc de Recerca Biomèdica de Barcelona. C/ Dr Aiguader, 88. 08003 Barcelona, España

An increasing perspective conceptualizes obesity and overeating as disorders related to addictive-like processes that could share common neurobiological mechanisms. We aimed at validating an animal model of eating addictive-like behavior in mice, based on the DSM-5 substance use disorder criteria, using operant conditioning maintained by highly palatable chocolate-flavored pellets. For this purpose, we evaluated persistence of food-seeking during a period of non-availability of food, motivation for food, and perseverance of responding when the reward was associated with a punishment. This model has allowed identifying extreme subpopulations of mice related to addictive-like behavior. We investigated in these subpopulations the Epigenetic and proteomic studies have allowed to identify a significant decrease in DNA methylation of CNR1 gene promoter in the prefrontal cortex of addict-like mice, which was associated with an upregulation of CB1 protein expression in the same brain area. The pharmacological blockade of CB1 receptor during the late training period reduced the percentage of mice that accomplished addiction criteria, which is in agreement with the reduced performance of CB1 knockout mice in this operant training. Proteomic studies have identified proteins differentially expressed in mice vulnerable or not to addictive-like behavior in the hippocampus, striatum, and prefrontal cortex. The use of DREADD techniques in this model has now allowed identifying the crucial role of the prefrontal cortex in the development of eating addictive-like behavior. This model provides an excellent tool to investigate the neurobiological mechanisms underlying eating addictive-like behavior.

S4 Room: Reading Room
THE MULTI-OSCILLATORY CIRCADIAN NETWORK: A KEY FOR PHYSIOLOGICAL PROCESSES AND HEALTH

Chair: Paul Pévet, FR

S4.1
GLUCOSE REGULATION: A REGULATORY FEEDBACK NETWORK OF PERIPHERY AND BRAIN

Andries Kalsbeek
Hypothalamic Integration Mechanisms, Netherlands Institute for Neuroscience (NIN), Meibergdreef 47, 1105 BA, Amsterdam, The Netherlands. Department of Endocrinology and Metabolism, Academic Medical Center (AMC) University of Amsterdam, Meibergdreef 9, 1105 AZ, Amsterdam, The Netherlands

Light is the most important cue for the brain biological clock in the suprachiasmatic nuclei (SCN) to synchronize its endogenously generated rhythmic activity with the outside world. The SCN master clock translates 'environmental time' to 'endogenous time' via hormones and the autonomic nervous system (ANS) to optimally prepare bodily physiology for recurring daily changes. The SCN directly controls (organs involved in) glucose homeostasis, including basal glucose levels and glucose tolerance.

Nowadays, electrical lighting is widely used around the world, but possible harmful effects of the 24/7 presence of light have become apparent only recently. In particular, use of light at night (LAN) is considered potentially disruptive, as this is an unnatural time for organisms to deal with light signals. LAN has been correlated with increased risk to develop cancer, depression, insomnia, overweight and obesity. Besides human epidemiology, rodents exposed to nocturnal (dim)light for several days or weeks, have been shown to increase body weight and develop glucose intolerance and insulin resistance. Acute experiments revealed alterations in the release of hormones, such as melatonin and corticosterone. Furthermore, 1-h of nocturnal light altered gene expression of clock and metabolic genes within multiple organs, including liver, pineal and heart. Surgical removal of the autonomic input to liver or adrenal reversed these effects of LAN, confirming the potential of light to affect the ANS.

The correlation between LAN and metabolic disorders together with the power of light to control the SCN and downstream targets, such as the liver, made us hypothesize that LAN might affect glucose homeostasis. We investigated this hypothesis by exposing Wistar rats to glucose and insulin tolerance tests at different times of day in abnormal lighting conditions. Light exposure at ZT15 and ZT21, as well as exposure to darkness at ZT3, acutely induced glucose intolerance. However, chronic exposure to continuous dim light at night did not affect glucose tolerance. The specific effects of the acute light exposures were dependent on time-of-day, resulting in increased glucose, but unaltered insulin responses at ZT15 and ZT3, and an increased insulin but unaltered glucose response at ZT21. Furthermore, we showed that at ZT15 the effect of light was dose dependent in terms of intensity and exposure duration. Currently we are investigating whether the effects of light are wavelength dependent. Additional experiments are aimed at revealing the mechanism of glucose tolerance, as insulin sensitivity was not affected and also changes in corticosterone and activity were not explanatory, as well as the neuro-anatomical substrate using co-staining's for c-fos and hypothalamic neuropeptides.

S4.2
THE MULTI-OSCILLATORY CIRCADIAN NETWORK: A KEY FOR PHYSIOLOGICAL PROCESSES AND HEALTH

Paul Pévet
Institute for Cellular and Integrative Neuroscience, CNRS, University of Strasbourg, Strasbourg, France

The molecular mechanism underlying the mammalian circadian clock, have been identified. Insight into how biological clocks are able to impose rhythmicity on our bodies has progressed enormously and disturbances of circadian rhythms have now been associated with numerous physiological disorders. The importance of circadian rhythmicity for human health and welfare is becoming increasingly recognized. The development of counteractive strategies to treat, prevent or delay such disturbance is a new challenge for science and medicine. Investigations of circadian cover all (behavioural, systemic, cellular and molecular) aspect of modern neurobiology. This system is thus a unique model for the study of brain functions in a vertical approach: the effect of environmental changes can indeed be recorded at the behavioural level in whole animals and then traced back to the neuronal circuits, their cellular components and finally to the molecular machinery of single cell.

The selected speakers are all well-known experts in one aspect or the other of the problematic. The combination of this expertise will permit to cover and discuss all new aspects of this rapidly changing field.

S4.3
RODENT MODELS TO STUDY THE EFFECTS OF DISTURBED CIRCADIAN RHYTHMS IN HUMANS

Etienne Challet
Regulation of circadian clocks Team, Institute of Cellular and Integrative Neurosciences, CNRS and University of Strasbourg, France

Many major biological functions are controlled by a complex circadian system synchronised by environmental factors such as light and food intake, whereas chronobiological alterations have potential pathogenic

consequences in humans. In particular, the increasing global reliance on shift-work (15–20% of the active population) has created major societal concerns, as large-scale epidemiological and experimental studies indicate that chronic shift-work is highly correlated with increased incidence of type 2 diabetes, obesity, and cardiovascular disease.

The master clock localized in the suprachiasmatic nucleus of the hypothalamus is mainly reset by light and controls the phase of secondary clocks in the brain and in peripheral organs, such as liver, heart and adrenal glands. These peripheral clocks can be shifted by meal time. By contrast, feeding signals do not affect markedly the master clock under light-dark conditions, although nutritional cues affect its functioning under metabolic challenges, such as calorie restriction and high-fat diet. Diet-induced obesity not only lengthens the period of the master clock, but also reduces its synchronization to light. The genetically obese (ob/ob) mice and severely diabetic (db/db) mice show differential alterations of photic synchronization. Exogenous leptin can act as chronomodulator in ob/ob mice by normalizing the phase-shifts induced by light. Chronic jet-lag in diurnal Grass rats not only causes a circadian desynchronization, but also glucose intolerance, indicating a pre-diabetic condition, as well as a shortening of telomeres, a biological marker of cellular aging. In conclusion; rodents are useful animal models to understand human pathophysiology associated with circadian desynchronization.

S4.4
SYNCHRONIZATION OF CLOCKS: THE AMBIENT TEMPERATURE, THE FORGOTTEN "ZEITGEBER" IN MAMMALS

Khalid El Allali
Hassan II Agronomy and Veterinary Institute, Comparative Anatomy Unit (URAC CNRST 49) and Medecine - and Surgical Unit of domestic animals, Rabat, Morocco

How the environmental light-dark cycle (LD cycle) is able to synchronize and to affect the circadian system at both molecular and physiological levels is now well documented. However, in mammals less is known about the ability of environmental cycles of ambient temperature (Ta) to entrain circadian rhythms. Indeed, since first experiments did not show a clear involvement of ambient temperature in the entrainment of circadian rhythms in some laboratory rodents, such environmental cues have not received much interest from researchers. In the present communication, we will show at least in desert mammals such as camel and goat, that the circadian timing system seems to be modulated by ambient temperature as well as by light-dark cycle. Depending on experiments, as a marker of activity of the clock, we have chosen at least two outputs of the clock: the daily rhythm of body temperature (Tb), the diurnal rhythm of locomotor activity (LA) and/or rhythm of plasma melatonin (Mel). In camel, we first demonstrated that the Tb rhythm is endogenous and under control of the circadian clock and also entrained by the LD cycle. Later, we demonstrated that this rhythm can be also entrained by the Ta cycle. Another known output of the master clock, the Mel rhythm, was monitored to ascertain that such effect is due to a real entraining capacity of the 24h Ta cycle on the master circadian clock and not due to a masking effect of thermoregulation. The obtained results demonstrated clearly that phase changes in the Ta cycle are able to shift not only the Tb rhythm, but also the Mel rhythm. The daily cycle of Ta can thus be considered in the camel, like the LD cycle, as a true Zeitgeber. To our knowledge, this was the first demonstration in mammals of such non photic entrainment on two circadian clock-outputs: the Tb and Mel rhythms. Thereafter, the question behind this finding was: is it a specific adaptation of the camel which can be related to its special heterothermy? Or is it a peculiarity of species living in the desert? The aim of next experiments was to verify such effects of Ta in another species of the desert, the local Moroccan goat. Here, we monitored three clock outputs rhythms, the daily rhythm of Tb, the diurnal rhythm of LA and the rhythm of Mel. Data show that as for the camel, the rhythm of Tb is an endogenous rhythm driven by the circadian clock (persistence under constant conditions) and synchronized by the LD cycle. Then, as in the camel, we demonstrated that by using of 24.0h artificial cycle of Ta and its reversal, we were able to induce in goat a real phase shift of the Tb rhythm. To rule out the possibility of a masking effect due to thermoregulation, we monitored in different experiments, two known robust outputs of the circadian system: Mel and LA rhythms. Mel rhythm is still under assay and data analysis. The preliminary results of LA show that it is a circadian rhythm since it persists under constant conditions and that a phase change in the Ta cycle is able to shift both the Tb rhythm and the rhythm of LA. The results are consistent with our hypothesis that beside photoperiod, the ambient temperature cycle is a synchronizer strong enough in arid and desert areas to affect the circadian system of mammals such as camel and goat. We conclude that for studies aiming to control circadian and seasonal rhythms (e.g. reproduction) in such species, not only photoperiod but also the Ta cycle have to be considered.

S4.5
CLOCKS WITHIN THE EYES, A KEY FOR A WELL-FUNCTIONING RETINA

Marie-Paule Felder-Schmittbuhl
Département de Neurobiologie des Rythmes, Institut des Neurosciences Cellulaires et Intégrative, CNRS UPR3212, Université de Strasbourg, Strasbourg, France. N-NRL laboratory, National Eye Institute, NIH, Bethesda, USA

Circadian rhythmicity is central to visual function by adapting retina physiology to the up to 10^6 fold changes in light intensity occurring over the 24h cycle. Indeed, many biological processes (expression of photopigments and phototransduction-related genes, visual sensitivity, light processing) as well as functions that are directly linked to retina survival (nocturnal release of the cytoprotective melatonin, photoreceptor outer segment phagocytosis, phototoxicity), are regulated on a daily pattern. The retina was the first circadian clock to be identified outside of the central clock of the suprachiasmatic nuclei, 20 years ago: it is able to sustain robust rhythms in melatonin release or clock gene expression in vitro. It is now known that integrity of the molecular clockwork is required for proper rhythmic function of the retina, even when exposed to the light/dark cycle, underlining the central role played by the circadian clock in retina physiology.

The retina displays extensive complexity, with many distinct cell types. Thus, one major challenge regards the identity of pacemaker cells as well as the mechanisms of synchronization between them. By using retina samples from mice carrying a knock in of luciferase reporter into the *Per2* clock gene, we show that each retina cell layer isolated by vibratome sectioning, is able to oscillate on its own, as assessed by real-time recording of emitted bioluminescence. We further demonstrate that the retina is a network of strongly coupled oscillators, in which gap junctions play a major role (Jaeger et al., 2015). Getting down to the cellular level, we characterize the molecular clockwork specifically expressed in rods, cones and Müller glial cells, as well as cognate clock target genes. We show that, while inner retinas are able to retain strong oscillatory capacity in vitro in the absence of photoreceptors, selective metabolic inhibition of glial cells totally blunts the capacity of the retina to show circadian rhythmicity. Thus Müller glial cells, which make contacts with a majority of cell types in the tissue, arise as a new central player in the retina multioscillatory system.

S4.6
MECHANISMS OF MAMMALIAN CLOCK FUNCTION IN NOCTURNAL AND DIURNAL SPECIES

Johanna H. Meijer
Leiden University Medical School, The Netherlands

Life on earth has developed under the evolutionary pressure of a light dark cycle. Most animals live in a world of light or a world of darkness, as in nocturnal (most standard laboratory animals) and diurnal (humans). The time of activity, be it nocturnal or diurnal, is controlled actively by the circadian timing system, and is synchronized to the environmental light-dark cycle.

Our biological rhythms are challenged in modern society by shift work, the 24-h economy and artificial light at night, all of which severely disrupt our biological clock. Studies from our and other labs have recently shown that attenuation of clock function is causally involved in the development of a wide variety of diseases, such as diabetes, obesity, sleep and mood disorders, cardiovascular disease, immune deficiencies, and muscle/bone retardation.

We record electrical activity (nerve impulse frequency) from the central neural clock (the SCN of the brain) of rodents, and this allows us to record the central SCN clock simultaneously with behaviour under various environmental manipulations (e.g., light/dark, photoperiod) and exercise/rest. We observed that the function of the key neurotransmitter GABA turns excitatory in long photoperiods. Given the increasing incidence of sleep, metabolic, and mood disorders in society, manipulation of light/exercise/GABA will be applied to boost the clock, and repair clock function in animals and humans with disrupted clocks. Applying the concepts of chronobiology to human health has been hampered by the fact that the clock mechanism has largely been investigated in nocturnal animals while people are diurnal. We have started investigations of the diurnal clock in order to develop strategies to manipulate, restore or even boost clock function in diurnal species, including humans.

S5 Room: Carlson Suite
GPCR HETERORECEPTOR COMPLEXES AND THEIR ADAPTOR PROTEINS IN PARKINSON'S DISEASE AND SCHIZOPHRENIA

Chair: Kjell Fuxe, SE

S5.1
GPCR HETERORECEPTOR COMPLEXES AND THEIR ADAPTOR PROTEINS GIVE NEW INTEGRAT-

IVE MECHANISMS THAT MAY GO WRONG IN PARKINSON'S DISEASE AND SCHIZOPHRENIA

Kjell Fuxe
Department of Neuroscience, Karolinska Institutet, Stockholm, Sweden

The receptor–receptor interaction field began with the studies on the neuropeptide/monoamine receptor–receptor interactions in membrane preparations in the early 1980s, which altered especially the affinity of the monoamine receptor subtypes. It was proposed that their allosteric interactions in the plasma membrane took place in postulated heteroreceptor complexes of GPCRs which could involve the participation of adaptor/scaffolding proteins. Now the receptor field in the CNS has expanded and includes not only monomers but also homo and heteroreceptor complexes representing receptor assemblies of unknown stoichiometry and geometry including adaptor proteins. They represent novel targets for treatment of neurological and mental diseases and give a new dimension to molecular neuroscience. Overall it appears that the integration of the signaling in homo and heteroreceptor complexes will have a major impact on medicine in general, and especially in the neurosciences. The heteroreceptor complexes in CNS are integrators of signaling. The integration of synaptic and volume transmission may take place through allosteric receptor-receptor interactions in heteroreceptor complexes. The same neural-glial network can produce differences in the balance of its various outputs. This happens when the diffusing VT-signals (glially and/or neuronally formed) by up-regulating or down-regulating synaptic strength, mainly through receptor-receptor interactions, changes the integrative action in different parts of the network. This will have a major impact on the flow of information through the brain circuits and thus on brain function and behaviors. The heterocomplexes represent major integrator centres in the plasma membrane and their protomers act as moonlighting proteins undergoing dynamic changes in their structure and function through the allosteric receptor-receptor interactions. In fact, we propose that the molecular basis of learning and memory can be based on the reorganization of multiples homo and heteroreceptor complexes into novel assembles in the post-junctional membranes of synapses giving them a new barcode.

The brain heteroreceptor complexes represent exciting new targets for neurotherapeutics in Parkinson's disease, schizophrenia, drug addiction, anxiety and depression. As one example, the antagonistic receptor-receptor interactions in A2A-D2 heteroreceptor complexes in the ventral and dorsal striatum should be mentioned which led to the introduction of A2A antagonists in the treatment of Parkinson's disease. They act in part by targeting the A2A-D2 heteroreceptor complexes in the dorsal striato-pallidal GABA neurons of the indirect pathway. Instead A2A agonists by acting inter alia at the A2A-D2 heteroreceptor complexes in the ventral striato-pallidal GABA anti-reward neurons may be novel atypical antipsychotic drugs for treatment of schizophrenia as well as novel anti-cocaine drugs for treatment of cocaine use disorder. However, it should be noticed that the D2R is a hub receptor capable of forming heteroreceptor complexes with more than ten different types of GPCRs in the neuronal-glial networks of the brain. This will increase its functional complexity but also its therapeutic potential through specific targeting of the different types of D2 heteroreceptor complexes through use of heterobivalent like compounds1.

S5.2
D2-DISC1 PROTEIN COMPLEXES AND THEIR RELEVANCE FOR SCHIZOPHRENIA

Fang Liu
Department of Neuroscience, Centre for Addiction and Mental Health, Toronto, ON M5T 1R8, Canada and Psychiatry, University of Toronto, Toronto, ON M5S 2J7, Canada

Current antipsychotic drugs primarily target dopamine D2 receptors (D2Rs), in conjunction with other receptors such as those for serotonin. However, these drugs have serious side effects such as extra-pyramidal symptoms (EPS) and diabetes. Identifying a specific D2R signaling pathway that could be targeted for antipsychotic effects, without inducing EPS, would be a significant improvement in the treatment of schizophrenia. We report here that the D2R forms a protein complex with Disrupted in Schizophrenia 1 (DISC1) that facilitates D2R-mediated glycogen synthase kinase (GSK)-3 signaling and inhibits agonist-induced D2R internalization. D2R-DISC1 complex levels are increased in conjunction with decreased GSK-3a/b (Ser21/9) phosphorylation in both postmortem brain tissue from schizophrenia patients and in Disc1-L100P mutant mice, an animal model with behavioral abnormalities related to schizophrenia. Administration of an interfering peptide that disrupts the D2R-DISC1 complex success-fully reverses behaviors relevant to schizophrenia but does not induce catalepsy, a strong predictor of EPS in humans.

S5.3
GPCR HETERORECEPTOR COMPLEXES IN MODELS OF PARKINSON'S DISEASE

Rafael Franco
Department of Biochemistry and Molecular Biology, University of Barcelona, Barcelona, Spain and

CIBERNED. Centro de investigación en red en enfermedades neurodegenerativas. Instituto de Salud Carlos III. Madrid. Spain

The hypothesis of direct interactions between pairs of G-protein-coupled receptors relevant for CNS function, launched by Luigi Agnati and Kjell Fuxe, has been confirmed and is now widely accepted. Interestingly, some of the firstly identified receptor heteromers (RHets) are expressed in the basal ganglia, inter alia adenosine A1 and dopamine D1 and adenosine A2A and dopamine D2 RHets. The GPCR more expressed in the CNS; the cannabinoid CB1, is also detected as forming RHets with A2A and D2 receptors. One of the main interest of RHets identification in striatum is their potential as targets combat anti-Parkinson's or other neurodegenerative diseases. One of the conditions for RHets being therapeutic targets is the persistence in pathological conditions. The talk will provide consistent data showing a variety of RHets that persist in the 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) primate and in the rodent models of Parkinson's disease. The effect of the most usual treatment, levodopa (L-DOPA), on the expression of receptors heteromers in the striatum will be also presented and discussed under the focus of progressive loss of therapeutic efficacy and/or the appearance of L-DOPA-induced involuntary movements.

S5.4
THE CELLULAR SCAFFOLD PROTEIN P11 STRONGLY MODULATES THE THERAPEUTIC EFFECTS OF L-DOPA IN PD. FOCAL INHIBITION OF P11 AS A NOVEL THERAPEUTIC TARGET

Per Svenningsson

Department of Clinical Neuroscience, Karolinska Institutet, Stockholm, Sweden

The reduced movement repertoire of Parkinson's disease (PD) is mainly due to degeneration of nigrostriatal dopamine neurons. Restoration of dopamine transmission by levodopa (L-DOPA) relieves motor symptoms of PD but often causes disabling dyskinesias. Subchronic L-DOPA increases levels of adaptor protein p11 (S100A10) in dopaminoceptive neurons of the striatum. Prior studies have also shown that p11 interacts with G protein coupled receptors (serotonin 1B and 4, metabotropic glutamate 5 and CC chemokine 10 receptors) and ion channels (including sodium channel Nav1.8, potassium channel subfamily K, acid-sensing ion channels, transient receptor potential cation channel subfamily V member 5) and increases their levels at the cell surface. P11 interacts with Using experimental mouse models of Parkinsonism, we report here that global p11 knockout (KO) mice develop fewer jaw tremors in response to tacrine. Following L-DOPA, global p11KO mice show reduced therapeutic responses on rotational motor sensitization, but also develop less dyskinetic side effects. Studies using conditional p11KO mice reveal that distinct cell populations mediate these therapeutic and side effects. Selective deletion of p11 in cholinergic acetyltransferase (ChAT) neurons reduces tacrine-induced tremor. Mice lacking p11 in dopamine D2R-containing neurons have a reduced response to L-DOPA on the therapeutic parameters, but develop dyskinetic side effects. In contrast, mice lacking p11 in dopamine D1R-containing neurons exhibit tremor and rotational responses toward L-DOPA, but develop less dyskinesia. Moreover, coadministration of rapamycin with L-DOPA counteracts L-DOPA-induced dyskinesias in wild-type mice, but not in mice lacking p11 in D1R-containing neurons. 6-OHDA lesioning causes an increase of evoked striatal glutamate release in wild type, but not in global p11KO mice, indicating that altered glutamate neurotransmission could contribute to the reduced L-DOPA responsivity. These data demonstrate that p11 located in ChAT or D2R-containing neurons is involved in regulating therapeutic actions in experimental PD, whereas p11 in D1R-containing neurons underlies the development of L-DOPA-induced dyskinesias. These changes of p11 may involve its interactions with serotonin 1B and metabotropic glutamate 5 receptors.

S5.5
A2A HETERORECEPTOR COMPLEXES AS THERAPEUTIC TARGETS IN PARKINSON'S DISEASE

Borroto Escuela Dasiel Oscar

Department of Neuroscience. Karolinska Institutet. Retzius väg 8. 17177 Stockholm, Sweden

The field of adenosine A2A heteroreceptor complexes in the Central Nervous System opens up a new understanding of the role of nondopaminergic receptors in Parkinson's diseases (PD). The hypothesis is given that changes in the function or balance of A2A homo and heteroreceptor complexes may help to understand the molecular mechanisms underlying the motor complications of long-term therapy in PD. Analysis of structural and functional A2A homo and heteroreceptor complexes (A2A-A2A, A2A-D2, A2A-mGlu5) was performed, upon degeneration of the striatal dopamine (DA) nerve terminal networks in models of PD, by means of in situ Proximity ligation assay. The analysis was performed in the dorsolateral striatum comparing the 6-OHDA lesioned side with the unlesioned side, 4 weeks after the lesion (6-OHDA microinjections into the medial forebrain bundle of the rat brain). The ratio between each heteroreceptor complex and the corresponding homoreceptor complex was of special interest to determine in

order to know if their balance was altered. The A2A-D2 heterocomplex in the dorsal striatum was found to be significantly increased on the lesioned side ($p < 0.05$, Student's paired t-test , $N = 3$ rats), which was true also for the A2A-mGlu5 heterocomplex ($p < 0.05$, Student's paired t-test , $N = 3$ rats). The A2A-A2A homocomplex was not significantly altered on the lesioned side versus the unlesioned side. Thus, the loss of DA terminals in the dorsal striatum on the lesioned side leads to an altered balance of the hetero and homoreceptor complexes with significant increases of the A2A-D2 and A2A-mGlu5 heterocomplexes on the lesioned side. In contrast, the A2A-A2A homocomplexes were not altered on the lesioned side versus the unlesioned side. These results may be interpreted as indicating that in the untreated hemiparkinsonian rat the A2A-D2 and A2A-mGlu5 heterocomplexes become more dominant favouring excitation of the dorsal striato-pallidal GABA neurons mediating motor inhibition.

S6 Room: Marie Louise 2 PAIN AND COMORBID MENTAL DISORDERS

Chair: Marc Landry, FR

S6.1 REWARD AND MOTIVATION IN PAIN AND PAIN RELIEF

Frank Porreca

Department of Pharmacology, University of Arizona, Tucson, AZ 85724 (USA)

Relief is a complex emotion that can result from both the termination of an aversive state, and activation of mechanisms that promote a positive hedonic state. Patients often perceive the relief of pain as rewarding and pleasurable. Primary rewards, or reward predicting cues, are encoded in brain reward/motivational circuits and considerable advances have been made in our understanding of neural signals and mechanisms underlying these rewards. On the other hand, the neural mechanisms underlying the hedonic and motivational features of pain relief are not known. Our work has explored the concept that the relief of pain is a natural reward that contributes to motivation and learning.

Pain is multidimensional with sensory, affective-motivational and cognitive domains all of which collectively elicit the human experience. Importantly, the affective or aversive features of pain are the main complaint of patients. Evaluation of pain in preclinical models has been limited by a relative inability to capture affective dimensions of pain in non-verbal animals. We have hypothesized pain can be unmasked in animals indirectly, by assessing the motivation to seek relief from pain aversiveness using the conditioned place preference (CPP) learning paradigm. Because ongoing pain provides a strong motivational drive to seek pain relief, motivational behavior in CPP paradigm can be utilized as an output measure of the efficacy of treatments to modulate pain aversiveness. Treatments that are clinically effective against ongoing pain in humans were also effective in the CPP paradigm while treatments ineffective in human are ineffective in CPP test. Thus, the CPP test correlated with clinical drug efficacy even in cases where evoked pain measures do not.

Studies using the CPP paradigm in rats are in agreement with the psychological investigations in humans that conceptualize relief of pain as a reward. Whether relief of pain with treatments that are not intrinsically rewarding results in the activation of mesolimbic reward circuits was investigated in several experimental pain models. For example, we showed that CPP induced by the treatment for post-operative pain (peripheral nerve block by popliteal fossa lidocaine injection) is accompanied by activation of dopaminergic neurons in the ventral tegmental area and an increase in dopamine efflux in the NAc shell. These effects were abolished by pharmacological blockade of dopaminergic signaling in the NAc. Thus, relief of pain aversiveness is reflected in activation of dopaminergic neurons of the mesolimbic reward system.

As opioids remain important treatments for relief of moderate to severe pain, we explored the role of endogenous opioid signaling in pain relief. The anterior cingulate cortex (ACC) has been implicated in pain aversiveness and expresses opioid receptors. Activation of opioid receptors in the ACC with morphine microinjection produced CPP and NAc DA release. These results suggested that activation of ACC opioid receptors is sufficient for the relief of pain aversiveness. Additionally, however, we showed that non-opioid pain relieving treatments (e.g., spinal clonidine, systemic gabapentin) require release of endogenous opioids in the ACC for the relief of pain aversiveness. These studies show that activation of opioid receptors in the ACC is also necessary for the relief of pain aversiveness. Thus, the relief of pain is encoded by opioid signaling in the anterior cingulate cortex, an area processing pain aversiveness, and dopamine signaling in the mesolimbic reward circuitry.

Release of endogenous opioids have been correlated with positive hedonic value supporting the conclusion that pain relief may result, in part, from neural processes that represent more than the termination of noxious stimuli.

S6.2 LONG-TERM CONSEQUENCES OF PERINATAL STRESS ON THE NOCICEPTIVE SYSTEM AND PAIN CONTROLS

Poisbeau Pierrick
Centre national de la recherche scientifique & Université de Strasbourg, Institut des Neurosciences Cellulaires et Intégratives, 5 rue Blaise Pascal, 67084 Strasbourg, France

Prematurity concerns as many as 1/10 birth in the world. Most of the time, they are submitted to repeated painful procedures in intensive care unit and as well as to many other non-painful stressors such as maternal separation (NMS). These perinatal events are known to have detrimental effect on the development of the newborn, especially on brain maturation. Clinical data strongly suggests that premature babies becoming adults are, for example, at high risk of developing neuropathologies affecting cognitive functions, chronic anxiety, depressive states, chronic pain and visceral dysfunctions.

In this study, we have analyzed the consequences of NMS on the development and efficacy of opioidergic pain controls in rats. NMS was produced by separating Wistar rat pups from their mother 3 hours per day, between postnatal day 2 and 12 (P2-P12), mimicking some developmental and environmental components of preterm birth. Efficacy of the inhibitory controls was analyzed in vivo by using either electrophysiological techniques on anesthetized animals or behavioral procedures in freely-moving rats.

We first found that NMS rats are hypersensitive to mechanical and thermal hot stimulation at all ages studied (up to P100). Furthermore, analgesic efficacy resulting from the activation of opioidergic descending pathways is strongly reduced or lost at all ages tested. In sharp contrast with control rats (i.e. having no NMS history), NMS animals do not exhibit stress-induced analgesia (SIA) and show impaired diffuse noxious inhibitory controls (DNIC). Analysis of spinal cord transcripts for opioid receptors reveals that kappa-type opioid receptors (KOR) are predominantly expressed by NMS rats and this is further supported by the observed KOR gene hypomethylation. To functionally test this hypothesis, we attempted to rescue the phenotype by administering a KOR antagonist in the spinal cord. We found that the spinal injection not only transiently restored normal (i.e. similar to control rats) nociceptive thresholds but we could also observed SIA and recruit DNIC.

Altogether, behavioral and electrophysiological results strongly suggest that opioidergic inhibitory controls of pain are impaired in adult rats previously submitted to NMS. Lack of efficacy of opioids in non-painful and painful stress conditions may easily explain several maladaptive behaviors observed after NMS in these animals. The spinal mechanisms underlying these alterations are currently under investigation but may also affect supraspinal structures expressing opioid receptors.

S6.3
ALTERATIONS OF PAIN RESPONSE IN A PHARMACOLOGICAL MODEL OF AN ADHD MOUSE MODEL

Bouchatta Otmane
Lab Pharmacology, Neurobiology and Behavior (URAC-37), Faculty of Sciences, Cadi Ayyad University, Marrakech, Morocco and IINS, CNRS UMR 5297, Bordeaux University, Bordeaux, France

Attention-deficit/hyperactivity disorder (ADHD) is a common neurodevelopmental disorder characterized by impaired attention and hyperactivity-impulsivity1. Recent evidence pointed to pain hypersensitivity in adult with ADHD history, and suggests possible comorbidity of ADHD with pain.

The most widely used neurodevelopmental model of ADHD is obtained by lesioning neonatal brain systems with 6-hydroxydopamine (6-OHDA) in rodents. So far, only one study used such a model in mouse, and demonstrated hyperactivity. However, data regarding impulsivity or attentional deficits remain unavailable. 1) The first objective of our project is to assess thoroughly the symptoms of the 6-OHDA- mediated dopamine depletion model in mouse. The second objective is to analyse comorbid pain behavior in this model. Finally, we will highlight circuits and mechanisms underlying pain alterations in ADHD animal models.

Firstly, we generated a mouse model at P5 by neonatal disruption of central dopaminergic pathways with 6-OHDA. Successful lesions were confirmed by tyrosine hydroxylase (TH) immunohistochemistry. In order to assess ADHD-like symptoms, a comprehensive set of behavioral tests was performed. After weaning (P24), spontaneous activity (open field) was evaluated. At P40 (adulthood), behavioral and cognitive deficits were tested in all mice: anxiety (elevated plus maze), working memory (latent inhibition and novel object recognition), social interactions (resident–intruder test), and inattention and impulsivity (five-choice serial reaction time task test). We analyzed nociceptive responses to thermal and mechanical stimuli by using hot plate, acetone-evoked cooling and von-Frey filaments. We also evaluated ADHD-like symptoms in inflammatory pain conditions. Spinal neuron activity was further examined *in vivo* by unit recording after mechanical stimulus. We tested the hypothesis that descending controls are responsible for pain alterations through the modulation of spinal circuits. The anterior cingulate cortex (ACC) is at the cross-road of pain and ADHD disorders, being involved in inattentiveness, impulsiveness and anxiety, as well as sending projections to the dorsal horn of the spinal cord4. Therefore, we explored whether ACC is critically involved in the dysfunction of descending

controls in ADHD-like mice. For this purpose, we recorded ACC activity, and the effects of ACC activation on mechanical stimuli-evoked responses in spinal cord wide-dynamic-range (WDR) neurons.

Neonatal dopamine (DA) depletion resulted in behavioral characteristics similar to those seen in patients with ADHD. At P24, ADHD-like mice exhibit hyperactivity. At P40, ADHD-like mice show anxiety, antisocial behavior, increased aggressiveness, mildly impaired latent inhibition and short-term memory. We also demonstrated attention deficit and increased impulsivity in ADHD-like mice. Mice with neonatal dopamine depletion exhibited a marked increase in both thermal (heat and cold) and mechanical sensitivity. Dopamine depletion also increased pain sensitivity in persistent pain conditions, i.e. at 4 days after Complete Freund's Adjuvant injection in the hindpaw. Interestingly, ADHD symptoms were not modified in inflammatory conditions suggesting that ADHD influences pain sensitivity while the reverse was not true. Electrophysiological recordings showed increased neuronal activity in response to both innocuous and noxious stimuli only in the ADHD-like group. Moreover, our data indicated that ACC neurons are hyper- activated in ADHD-like mice. Finally, we found that the electrical stimulation of controlateral ACC (100 Hz; 10, 20, and 30 V; 10 s) increases the responses (amplitude and mean spike frequency) of WDR neurons to innocuous and noxious stimuli.

Our results demonstrated the validity of the neonatal 6-OHDA model to mimic ADHD syndrome. Taken together, our data demonstrate that ADHD conditions induce WDR spinal cord neurons hyperactivation and pain hypersensitivity. We also suggest that the deregulation of ACC may be the trigger for spinal neuron dysfunction.

S6.4
NEURAL SITES AND MECHANISMS MEDIATING RECIPROCAL INTERACTIONS BETWEEN PAIN AND NEGATIVE AFFECT

David P. Finn
Pharmacology and Therapeutics, Galway Neuroscience Centre and Centre for Pain Research, NCBES, National University of Ireland Galway, University Road, Galway, Ireland

Pain and affective state interact reciprocally, whereby the latter can both influence, and be influenced by, the pain experience. We have used animal models to elucidate supraspinal neurochemical and receptor mechanisms involved in (1) hyperalgesia associated with negative affect (anxiety/depression) and (2) fear-induced analgesia. Wistar-Kyoto rats exhibit an anxiety- and depressive-like phenotype and also display hyperresponsivity to noxious stimuli. These effects are associated with alterations in levels of endogenous cannabinoids (endocannabinoids) and related N-acylethanolamines and altered expression of their receptor targets or metabolizing enzymes in key brain regions regulating pain and affect. Pharmacological blockade of the CB1 receptor exacerbates hyperalgesia to persistent inflammatory pain in Wistar-Kyoto rats, while pharmacological blockade of endocannabinoid degradation attenuates hyperalgesia. Additional data suggest an important role for the endocannabinoid system in the periaqueductal grey and rostral ventromedial medulla in regulating hyperalgesia in the Wistar-Kyoto model of hyperalgesia associated with negative affective state. Our most recent results also suggest an important role for TRPV1 and PPAR in the periaqueductal grey in the Wistar-Kyoto model. Further evidence that deficits in the functionality of the descending inhibitory pain pathway likely underlie the hyperalgesic phenotype of Wistar-Kyoto rats comes from our recent data suggesting that these rats exhibit impaired expression of fear-induced analgesia. Interestingly, we have also shown that induction of neuropathic pain in the Wistar-Kyoto rat (L5 spinal nerve ligation) is associated with significantly increased anxiety- and depressive-like behaviour compared with Sprague-Dawley counterparts, results which may be due, at least in part, to deficits in endocannabinoid signalling. This result maps onto clinical data that we and others have generated indicating increased anxiety and depression in neuropathic pain patients. Our work also points to a role for non-CB1 receptor targets of endocannabinoids and N-acylethanolamines in the affective dimension of pain, particularly in higher brain centres including the medial prefrontal cortex. These targets include peroxisome proliferator activated receptors (PPARs) and GPR55. Finally, using the place escape avoidance paradigm we have generated evidence for an affective component associated with our recently developed novel rat model of post-operative pain associated with inguinal hernia repair and we are elucidating the role of the endocannabinoid system in the affective dimension of post-operative pain in both rodents and humans. Increased understanding of the neurochemical and receptor mechanisms underpinning pain-affect interactions may facilitate identification of novel therapeutic targets for the treatment of pain, affective disorders, and their co-morbidity.

S6.5
INTRA-AMYGDALA CHOLECYSTOKININ MODULATES INFLAMMATORY PAIN

Olivier Roca-Lapirot
University of Bordeaux, IINS, UMR 5297, Bordeaux, France and CNRS, IINS, UMR 5297, Bordeaux, France

Pain and emotion has long been considered in a close relationship. Anxiety, as a negative emotion is a consequence of chronic pain but might have a causal role in pain worsening as well. Choleycstokinin (CCK) is an anxiety-inducing peptide when it is released into the central nucleus of the amygdala (CeA). It is also known to play a role in pain descending facilitation. Thus CCK is at a crossroad between pain and emotion. In this study, we aimed at investigating the effect of intra-CeA CCK on pain thresholds and nociceptive integration into the spinal cord, in a rat model of inflammatory pain induced by complete Freund's adjuvant (CFA). We showed that intra-CeA infusion of CCK induced both an antiallodynic effect on awake animals and a reduction of nociceptive activity in spinal dorsal horn neurons, only in animals submitted to inflammatory pain. These analgesic effects are accompanied by CCK- induced increase of CeA neuron excitability as recorded in slices from inflammatory rats. Such changes in CCK-induced modulation of CeA neuron activity are supported by plasticity of the CCKergic system in the CeA. CCK1 but not CCK2 receptor mRNAs are specifically downregulated in inflamed rats while CCK immuno-reactivity is increased. We hypothesized that intra-CeA CCK triggers the activation of pain descending control through the midbrain periaqueductal grey (PAG). Thus we assessed the effect of blocking PAG activity on CCK antinociceptive effect. The results showed that after infusing lidocaine into PAG, CCK infusion into CeA had no more effect on spinal nociceptive activity. These results were supported by investigating the anatomical relationships between CCK fibers and retrogradely traced efferent neurons projecting from CeA to PAG. This showed a high level of appositions between CCK terminals and retrogradely traced neurons which were observed by confocal microscopy. All these results showed for the first time (i) an antinociceptive effect of CCK, (ii) changes of intra-CeA CCK functions in inflammatory pain condition and (iii) potential effect of intra-CeA release of CCK in activation of a PAG-dependant descending inhibitory control that reduces the excitability of nociceptive spinal neurons and hence ultimately alleviates pain behaviour.

S7 Room: Ballroom
5-HT_{2C} RECEPTORS: WIDESPREAD CONTROL OF NEUROBIOLOGICAL NETWORKS: I

Chair: Giuseppe Di Giovanni, MT

S7.1
COMPARATIVE ANALYSIS ON 5-HT_{2C}, 5-HT_{1A} AND 5-HT_7 RECEPTORS ON THEIR ROLE IN ANXIETY AND DEPRESSION

Evgeni Ponimaskin
Cellular Neurophysiology, Center of Physiology, Hannover Medical School, Carl-Neuberg-Straße 1, 30625 Hannover, Germany

Serotonin receptors 5-HT_{2C}, 5-HT_{1A} and 5-HT_7 are highly co-expressed in brain regions implicated in depression. However, their functional interaction has not been established. We have recently demonstrated that acute administration of 5-HT_{2C} receptor agonist caused significant dose-dependent reduction of locomotor activity in mice. In addition, we compared 5-HT_{2C} receptor-induced reduction of locomotor activity in seven different inbred mouse strains and found the considerable difference in genetically defined 5-HT_{2C} receptor functional response. We also obtained the interstrain correlation of 5-HT_{2C} receptor-mediated hypolocomotion and 5-HT_{2A} receptor-mediated head-twitches number, suggesting the shared mechanisms for the regulation of 5-HT_{2C} and 5-HT_{2A} receptors functional responses.

In addition, we have shown that 5-HT_{1A} and 5-HT_7 receptors form heterodimers both *in vitro* and *in vivo*. Functionally, heterodimerization is critically involved in initiation of the serotonin-mediated 5-HT_{1A} receptor internalization and markedly decreases the ability of the 5-HT_{1A} receptor to activate G-protein gated inwardly rectifying potassium channels in hippocampal neurons, demonstrating a physiological relevance of heteromerization *in vivo*.

To investigate the role of heterodimerization on G-protein mediated signalling, we combined online oligomerization analysis by 'linear unmixing FRET' (luxFRET) with the simultaneous measurement of [cAMP] by application of FRET based biosensors. Based on such measurements we developed a biophysical model describing correlation between the cAMP level and extend of receptor-receptor interaction. This model allows to estimate the ratio of endogenous expression level of 5-HT_{1A}R and 5-HT_7R in hippocampal neurons at defined developmental stages.

Generally, our data suggest that the regulated and balanced ratio of homo- and heterodimerization on pre- and postsynaptic neurons may be critically involved in the onset of psychiatric diseases (e.g. depression and anxiety) and addiction.

S7.2
IDENTIFICATION OF THE SUBSET OF 5-HT_{2C}RS THAT CONTROL APPETITE AND IMPROVE OBESITY AND TYPE 2 DIABETES

Lora K. Heisler
Rowett Institute of Nutrition and Health, University of Aberdeen, Aberdeen, UK

The 5-hydroxytryptamine (5-HT, serotonin) 2C re-

ceptor (*Htr2c*; 5-HT_{2C}R) agonist lorcaserin (Arena Pharmaceuticals) is a new medication for obesity that we report also improves a type 2 diabetes (T2D) phenotype in mice. However, the neural circuits mediating lorcaserin's therapeutic effects remain to be elucidated. We observed that preventing *Pro-opiomelanocortin* (*Pomc*) expression ($Pomc^{NEO}$) within the arcuate nucleus of the hypothalamus (ARC) abolished lorcaserin's anorectic and glucoregulatory effects and that restoration of Pomc specifically within a subset of ARC neurons expressing 5-HT_{2C}Rs ($Pomc^{Htr2c}$) is sufficient to mediate lorcaserin's therapeutic effects. Using a combination of viral technology and genetics, we reveal a downstream circuit through which lorcaserin influences appetite, but not glycaemic effects. Specifically, lorcaserin suppresses appetite via downstream melanocortin4 receptors (*Mc4r*). Moreover, brain-derived neurotrophic factor (*Bdnf*) neurons within the ventromedial nucleus of the hypothalamus ($Bdnf^{VMN}$) mediate lorcaserin's anorectic effect in a melanocortin4 receptor (*Mc4r*)-dependent manner. We next investigated the circuit through which lorcaserin influences glucose homeostasis. We observed that lorcaserin improves insulin sensitivity and suppresses hepatic glucose production (HGP). We next determined that lorcaserin requires functional *Mc4rs* to elicit glycaemic effects. On a *Mc4r*null background, the selective restoration of *Mc4r* function within ChAT neurons ($Mc4r^{ChAT}$) is sufficient to mediate lorcaserin's glucoregulatory, but not anorectic effects. Thus, our results reveal a mechanism through which lorcaserin improves both obesity and T2D in mice. The subset of 5-HT_{2C}Rs that are specifically expressed within the ARC on POMC neurons mediate both lorcaserin's appetitive and glucoregulatory effects. However, these POMC-5-HT_{2C}R expressing cells signal to a Mc4r to Bdnf circuit to influence appetite whereas they signal to a $Mc4r^{ChAT}$ circuit to influence glucose homeostasis. These findings suggest that 5-HT_{2C}R agonists can promote an improvement in both obesity and type 2 diabetes, via divergent downstream circuits.

S7.3
SEROTONIN 5-HT_{2C} RECEPTOR POSITIVE ALLOSTERIC MODULATORS: POTENTIAL FOR THERAPEUTICS IN ADDICTIVE DISORDERS

Kathryn A. Cunningham
Center for Addiction Research and Department of Pharmacology and Toxicology University of Texas Medical Branch Galveston, Texas, USA

An impaired signaling capacity of the serotonin (5-HT) 5-HT_{2C} receptor (5-HT_{2C}R) contributes to the neurobehavioral processes that underlie addictive disorders as well as other chronic health issues (e.g., depression, impulsivity disorders, obesity). We propose that restoration of the diminished signaling capacity in a site- and event-specific manner may be achievable through positive allosteric 5-HT_{2C}R modulators (PAMs). Based upon the structure of a small molecule (PNU-69176E) which was found to exhibit the profile of a 5-HT_{2C}R PAM, we optimized the synthetic route to readily access PNU-69176E and its diastereomer in good overall yields. Our team has recently described our efforts in the rational design, chemical synthesis, and pharmacological evaluation of novel, simplified 5-HT_{2C}R PAMs based on PNU-69176E as the chemical lead. Multiple analogues have been shown to potentiate 5-HT-evoked intracellular calcium release (Cai^{++}) in Chinese Hamster Ovary (CHO) cells stably expressing the human 5-HT_{2C}R (h5-HT_{2C}R-CHO), but not in h5-HT_{2A}R-CHO cells. Our lead compound CYD-1-79 potentiated 5-HT_{2C}R-induced Cai^{++} release ($p < 0.05$) and activation ERK1/2 ($p < 0.05$), but did not exhibit intrinsic activity when tested alone in stably-transfected 5-HT_{2C}R-CHO or h5-HT2AR-CHO cells. CYD-1-79 also exhibited a favorable overall pharmacokinetic and behavioral profile in rodent models of impulsivity and addiction in rats. Taken together, these data represent the only combination of *in vitro* and *in vivo* evidence of synthetic small molecules acting as 5-HT_{2C}R PAMs, providing a proof of concept that allosteric modulation of 5-HT_{2C}R may be a viable strategy toward the discovery of novel neurotherapeutics. Optimization of our newly-identified 5-HT_{2C}R PAMs and further evaluation of these molecules in preclinical models will allow us to develop novel pharmacotherapies for addictive and other chronic health disorders.

S8 Room: Clermont Suite
NEUROINFLAMMATION: DISEASE MODELS, MOLECULAR AND CELLULAR MARKERS, *IN VIVO* MONITORING

Chairs: Klaus G. Petry, FR and Sylvie Chalon, FR

S8.1
THE IDENTIFICATION OF PEPTIDE BIOMARKERS MIMICKING THE COMPLEXITY OF PROTEIN INTERACTIONS AT THE BLOOD BRAIN BARRIER IN EXPERIMENTAL MULTIPLE SCLEROSIS

Klaus G. Petry
INSERM U1029 – Bordeaux University, 33615 PESSAC France

In Multiple Sclerosis (MS) neuroinflammation pathology, complex molecular alterations at the blood brain

barrier (BBB) are permissive to the CNS infiltration of blood born immune cells and circulating compounds. To better understand the neuroinflammation processes, characterizing the complexity of protein interactions specific to such alterations is necessary. We choose to identify peptide ligands reacting with the altered BBB by applying *in vivo* phage displayed 12aa peptide screening in CNS neuroinflammation of the experimental autoimmune encephalomyelitis (EAE) rat model of MS and healthy controls. We pursued two major goals: 1) analyzing the generated peptide repertoires to gain new insights into the complexity of protein interactions in neuroinflammation, 2) identifying specific targeting agents among the obtained peptides and their defined protein interactions. We performed peptide phage display without *a priori* knowledge of target molecules generating massive peptide repertoires in both EAE and healthy rats. Random picking of peptides expressing phage clones revealed no discrimination between EAE pathology and not affected CNS. Indeed, massive NGS data analysis confirmed a large spectrum of common peptides among both repertoires. To overcome this problem of not-disposing specific peptide ligands for biotesting, we developed a novel DNA subtraction method eliminating 95% of phage clones expressing peptides that are common to both repertoires. The generated subtraction repertoire provided first EAE specific biomarkers, particularly reacting with vascular targets of inflamed BBB. Further bioinformics developments allowed defining clusters of peptides mimicking protein segments of the human and mouse genome. We evaluated the importance of mimicking peptides by applying high significance of retained peptide clusters distribution between the peptide repertoires of EAE, healthy and random controls (5 sigma, no background) allowing the identification of the mimicked proteins. Bioinformatics interactom analysis of these proteins using Stranger "reactom" algorithm of experimentally confirmed interactions revealed known functions, including inflammation-immune cell activation, axonal repair, cell signaling, matrix modulation etc, which confirmed the mimicked known processes in neuroinflammation. However, many of the mimicked proteins were not considered in neuroinflammation pathology. Among the most importantly ranked mimicked proteins, we then focused on an 18aa mimicked protein segment that is rare in the mammalian genome. Experimentally, in neuroinflammation we tested this synthesized human protein segment (biotinylated peptide) in histology on EAE and MS CNS sections. The biotinylated peptide specifically labeled the vasculature (endothelium) at the inflamed BBB (characterized by perivascular immune cell infiltrates) and reveals no binding to healthy CNS. For *in vivo* studies, the identified peptide and as control a scrambled peptide (same size and aa composition) were covalently linked to a nanocarrier containing a MRI contrast agent and a fluorescent dye. By introducing a needle wound lesion (IL-1β injection into striatum) in rat CNS, the nanocarrier, upon intravenous injection, was guided by the peptide to accumulate in the injection area. Fluorescence histology confirmed the endothelial peptide labelling at the BBB. To further evaluate the biotinylated peptide binding to endothelial cells in relation to inflammatory processes in the human CNS, we used the human adult brain endothelial cell line hCMEC/D3 as BBB model. Besides presenting major BBB properties under resting conditions, the model responds to proinflammatory conditions (IL-1β) by inflammatory cytokine expression and tight junction disruption. Experiments with the synthetized biotinylated peptide on hCMEC/D3 revealed specific and strong binding under IL-1β activation.

In conclusion, we have identified a battery of peptides specifically binding to cellular and molecular BBB alterations in neuroinflammation. They mimic interaction sites of identified proteins. These proteins could be expressed by immune cells, but potentially some of them could also be released into blood circulation from peripheral organs.

S8.2
PET IMAGING OF TSPO TO MONITOR MICROGLIA ACTIVATION AND THEIR RELEVANCE TO DISEASE SEVERITY PROGRESSION IN MODELS OF SUBARACHNOID HEMORRHAGE, ALZHEIMER'S, AND PARKINSON'S DISEASE

Sylvie Chalon
UMR Inserm U930, Université François Rabelais de Tours, Tours, France; Faculty of Health Sciences, School of Chemistry, University of Sydney, Sydney, Australia

Neuroinflammation plays a major role in a number of acute and chronic brain disorders. This process is especially characterized by the activation of microglia cells, including changes in their morphology, migration, proliferation, and capacity to express and release proinflammatory molecules. The mitochondrial translocator protein 18 kDa (TSPO), formerly known as the peripheral benzodiazepine receptor is one of the earliest molecules overexpressed by the activated microglia. *In vivo* brain exploration of this molecular target is therefore a potent and sensitive marker for the detection and follow-up of neuroinflammation. Several radiotracers are to date available for TSPO exploration by positron emission tomography (PET) such as the reference ligand [^{11}C]PK-11195 and more recently other compounds labeled with fluor-18, which has the advantage

of a longer half-life (i.e. 110 vs 20 min). Among these tracers, [^{18}F]DPA-714 is available both for preclinical and clinical studies. We used this tracer in a rat model of acute brain lesion, i.e. subarachnoid hemorrhage (SAH), and two rodent models of neurodegenerative affections, i.e. Alzheimer's and Parkinson's disease.

We used a rat model of SAH in which different degrees of bleeding can be obtained, graded from 0 to 12 (according to the Sugawara method). We observed that at 48 h post-surgery, an increased accumulation of [^{18}F]DPA-714 occurred on the lesioned hemisphere, and demonstrated that the intensity of the signal in the striatum was strongly and positively correlated with the SAH grade. We confirmed the microglial activation assessed by PET imaging through complementary *in vitro* methods such as autoradiography (with [^{3}H]PK-11195) and immunohistochemistry (CD11b and GFAP immunostaining). These preclinical data suggested that PET exploration of neuroinflammation could be used in human as relevant index of the severity of SAH and of the risk of unfavorable secondary evolution such as delayed cerebral ischemia.

In the APPswePS1dE9 transgenic mouse model of Alzheimer's disease, we followed longitudinally in each Tg and WT animal the density of TSPO and beta-amyloid plaques using PET imaging with [^{18}F]DPA-714 and [^{18}F]AV-45 (florbetapir), respectively. Each animal was explored at 6, 9, 12, 15 and 19 months of age. We determined that the main affected brain areas were the cortex and hippocampus. In addition, PET imaging data obtained with both tracers were positively correlated, and were also in accordance with complementary *ex vivo* data (autoradiography, immunohistochemistry) explored at the latest age. These results highlight the usefulness of PET imaging in Alzheimer's disease and were in agreement with the hypothesis of a "vicious circle" between the amyloid load and inflammation during the pathological process.

In a rat model of Parkinson's disease induced by unilateral striatal administration of 6-hydroxydopamine, we explored in same animals the neuroinflammation and neurodegenerative processes. This was achieved through PET imaging of the TSPO and the dopamine transporter (DAT) as a relevant marker of dopaminergic nerve endings, using [^{18}F]DPA-714 and [^{18}F]LBT-999, respectively. We observed that the increase in the TSPO marker and the decrease in the DAT marker were correlated, suggesting a strong interaction between neuroinflammation and neurodegeneration. In addition, the administration of an alpha-7 nicotinic receptor agonist in lesioned rats lead to a concomitant reduction of the TSPO marker and increase of the DAT marker.

These whole preclinical results of PET imaging of neuroinflammation through the marker TSPO demonstrate the usefulness of this in vivo approach, especially as a predictive indicator of the evolution of the disease, and as a relevant index of the evolution of neuroinflammation and neurodegeneration. These both aspects of exploration can be highly helpful in human applications, and can also bring the opportunity to longitudinally evaluate the effects of potential treatments.

S8.3
THE MONOCYTES RECRUITMENT IN STROKE AFFECTION AND THEIR PHENOTYPE MODULATION IN CNS REPAIR

Anna M. Planas
Institut d'Investigacions Biomèdiques de Barcelona (IIBB), Spanish Research Council (CSIC), Institut d'Investigaciones Biomèdiques August Pi i Sunyer (IDIBAPS)

Stroke triggers an acute inflammatory reaction involving local microglia reactivity and leukocyte infiltration. It also induces a plethora of peripheral effects, including an increased release of immature pro-inflammatory monocytes from the bone marrow to the circulation. Leukocytes are attracted by chemokines produced in the injured brain tissue. The perivascular spaces and the meninges normally contain macrophages (PVMM) that are believed to have immune surveillance functions. We interrogated the function of PVMM in the first hours after stroke using strategies allowing the depletion of this cell population. Circulating monocytes reach the injured brain tissue where they become tissue macrophages, which have many markers in common with reactive microglia and the distinction between these cells of different origin is difficult. By using strategies of adoptive transfer of reporter monocytes and generating reporter chimeric mice with fluorescent peripheral myeloid cells, we found that macrophages invade the core of the lesion while ramified reactive microglia surround the ischemic core 4 and 7 days after brain ischemia. Microglia and macrophages can acquire various phenotypes and have functional diversity. Following stroke, infiltrating monocytes become tissue macrophages and acquire the expression of certain markers of alternative activation, such as arginase-1 and YM-1, suggesting a role in clearing the damaged brain tissue paving the way for tissue repair. In contrast, markers of alternative activation are hardly observed in the reactive microglial cells located at the periphery of infarction, suggesting differential functions of microglia and infiltrating macrophages. Microglial cells are able to proliferate and have powerful phagocytic activity. We found that microglial cells are able to phagocyte the leukocytes that reach the periphery of the lesion, notably neutrophils, in agreement with a recent report. Therefore, microglial cells generate a barrier that prevents the in-

vasion of infiltrating neutrophils and monocytes beyond the core of the infarction. These cells can upregulate the expression of MHCII and can present antigen, yet with lower efficiency than the prototypical antigen presenting cells, i.e. dendritic cells. Cells expressing CD11c, a typical marker of conventional dendritic cells, are also found in the ischemic brain but whether they are different than activated microglia or macrophages is still a matter of debate. We have sorted the different myeloid cell populations and studied the gene expression profiles and their response to brain ischemia. Unraveling the precise functions of all these cells together with their selective molecular determinants might have therapeutic implications.

S8.4
MRI MODALITIES TO INVESTIGATE NEUROINFLAMMATION IN EXPERIMENTAL AND CLINICAL STUDIES

Mario Quarantelli
Biostructure and Bioimaging Institute, National Research Council, Naples, Italy

Non-invasive techniques that allow to study *in vivo* the complex cascades of events involved in different aspects of neuroinflammation are increasingly required, as a consequence of the growing interest in the role of neuroinflammatory phenomena, also in pathologies (e.g. in Alzheimer's disease or in psychiatric disorders) not traditionally considered as primarily neuroinflammatory.

Magnetic Resonance techniques allow to study most mechanisms underlying the brain/immune interaction, thus assessing several aspects of neuroinflammation, using a heterogeneous host of complementary approaches to probe the mechanisms underlying the brain/immune interaction at the level of vascular, cellular and interstitial compartments.

While the advantages of these techniques include their large availability and the lack of ionizing radiations, they are still limited by a relatively low specificity (especially when compared to nuclear medicine techniques that allow a both sensitive and specific detection of microglial activation).

Despite these limitations, MRI is helping unravel the multi-faceted role of neuroinflammation in several CNS diseases.

We will review MRI methods that have been used to assess neuroinflammatory phenomena in preclinical and clinical studies, grouped according to the neuroinflammatory processes that they probe. Accordingly, MRI methods will be discussed that are suitable to monitor the neuroinflammatory phenomena assessing: endothelial activation (by USPIO-labelled antibodies against surface molecules); BBB permeability alterations (by dynamic contrast-enhanced MRI); intercellular compartment modifications (by MR spectroscopy, diffusion or magnetization-transfer imaging); immune cell trafficking (using iron oxide colloids). In addition, MR spectroscopy (MRS) markers of glial activation and of immune cell activity will be considered.

Finally, conventional and advanced MRI techniques used to assess the sequelae of neuroinflammation, including demyelination, neuronal/axonal loss, and demyelination/gliosis, will be examined.

For each technique, main advantages and limitations will be debated, also in view of their potential clinical exploitation, discussing their currently ongoing transition from pre-clinical to clinical applications, which has the potential to introduce significant changes in the diagnosis and management of several neurological diseases, not limited to the neuroinflammatory ones.

S9 Room: Carlson Suite
TRANSCRANIAL MAGNETIC STIMULATION: RE-WIRING THE ADDICTED BRAIN

Chair: Marco Diana, IT

S9.1
EXPLOITING THE HYPODOPAMINERGIC STATE WITH TMS IN ADDICTS: PRELIMINARY OBSERVATIONS

Marco Diana
'G. Minardi' Laboratory of Cognitive Neuroscience, Dept. Chemistry and Pharmacy, University of Sassari, Italy

Repetitive Transcranial Magnetic Stimulation (rTMS) of the dorsolateral prefrontal cortex may affect neuro-adaptations associated with alcohol addiction, potentially influencing drug craving and intake. Previous pre-clinical and clinical evidence suggest a tonically reduced functioning of the mesolimbic dopamine system leading to hypothesize that 'boosting' the hypofunctional system may yield clinical benefits. Here we show that rTMS reduces alcohol and cocaine intake in alcoholics and cocaine addicts. We investigated alcohol intake and dopamine transporter (DAT) availability by Single Photon Emission Computed Tomography (SPECT) in the striatum, in Alcohol Use Disorder (AUD) patients before and after deep rTMS. Fourteen patients underwent baseline clinical and SPECT assessment. Eleven out of 14 patients were randomized into two groups for the REAL or SHAM treatment. Clinical and SPECT evaluations were then carried out after four weeks of rTMS sessions. At baseline, AUD patients showed higher striatal DAT availability than healthy control subjects (HC). Further, patients receiving the REAL stimulation revealed a reduction

in DAT availability, whereas SHAM-treated did not. In addition, REAL patients decreased alcohol intake and state anxiety levels. The present results suggest a modulatory effect of deep rTMS on dopaminergic terminals and a potential clinical efficacy in reducing alcohol intake in AUD patients.

Similarly, 18 cocaine addicts (DSM-V) were admitted and randomly assigned to the active or sham stimulation protocol in a double-blind experimental design. They received 12 repetitive TMS r(TMS) sessions 3 times a week for 4 weeks at 100% of motor threshold, over bilateral DLPfc. Cocaine intake (ng/mg) was assessed by hair analysis at baseline (before treatment, T0), after one month (end of treatment, T1) and at 3 (T2) and 6 (T3) months later. All subjects received weekly psychological support. Bilateral TMS of the DLPfC produces a lasting reduction of cocaine-intake significantly more in 10 Hz treated patients vs. SHAM. While further studies are required to confirm these encouraging, preliminary findings they support the notion that DA can be considered a useful biomarker to be targeted by rTMS in addicts.

S9.2
EVALUATING DEEP TMS (DTMS) TARGETING THE INSULA AS A POTENTIAL TREATMENT FOR ALCOHOL DEPENDENCE: DESIGN ISSUES AND INTERIM RESULTS

Markus Heilig
Center for Social and Affective Neuroscience, Linköping Univ, Sweden

The insula, a distinct lobe situated in the depth of the Sylvian fissure, is activated by aversive interoceptive stimuli such as pain or nausea. Its activity has i.a. been hypothesized to encode "homeostatic emotions", i.e. emotional error signals that promote behaviors aimed at restoring a desirable body state. In addiction, activity of the insula is closely correlated with the subjective experience of craving. This association may be causal, since stroke resulting in loss of insular cortex has been reported to allow smokers to quit without experiencing urges to smoke.

Development of the H-coil by Zangen and colleagues has allowed TMS to target brain structures located up to appr. 4 cm beneath the skulle surface, putting the insula within reach of stimulation. A study by Zangen and collaborators then provided support for dTMS targeting the insula to promote smoking cessation. Inspired by these findings, we have collaborated with the Zangen group to develop and implement a set of randomized, sham controlled trials targeting the insula and the Anterior Cingulate Cortex (ACC), respectively, for the treatment of alcohol dependence.

Device-based interventions such as TMS pose particular challenges when it comes to developing study designs able to establish causal, mechanism-based therapeutic actions. We will discuss several of these challenges, such as the appropriate choice of comparator conditions to control for the potent placebo effects observed with TMS, and for site specificity. We will further describe a set of imaging-based biomarkers used in our work to establish the equivalent of what in pharmacotherapy trials would be referred to as target engagement. Finally, we will provide interim analyses of biomarkers, CSF analyses and clinical outcomes.

S9.3
ELECTROMAGNETIC STIMULATION IN THE STUDY AND TREATMENT OF ADDICTION: FROM ANIMAL MODELS TO HUMAN APPLICATIONS

Abraham Zangen
Department of Life Sciences and the Zlotwosky Center for Neuroscience, Ben-Gurion University, Beer Sheva, Israel

The pathophysiology of addiction involves impaired excitability and function of reward-related circuitries. Repeated electromagnetic stimulation of these circuitries can induce lasting alterations in excitability and function of these networks, thereby becoming a potential therapeutic approach. Our animal studies revealed that multiple sessions of localized stimulation of the prefrontal cortex can alter molecular and behavioral features of cocaine addiction. In order to affect the relevant circuitries without a surgery, we have designed special transcranial magnetic stimulation (TMS) coils that enable stimulation of much deeper regions relative to those directly affected by conventional TMS coils. These coils, termed H-coils, were tested for their safety and ability to reach deeper brain regions, and evaluation of their antidepressant potential when applied over the prefrontal cortex of medication-resistant depressive patients showed high rates of remission in a large multicenter study. The therapeutic potential of other versions of H-coils are evaluated in several psychiatric disorders including addiction. The use of an H-coil version targeting the prefrontal and insular cortices in heavy smokers showed effectiveness when high, but not low frequency was applied, especially when combined with activation of the craving-related circuitries by presentation of smoking cues just prior each stimulation session. This study led to an ongoing large multi-center sham-controlled study, now taking place in 15 centers, primarily in the united states. Deep TMS is a relatively novel tool in psychiatric and basic brain research. The ability to induce non-surgical direct stimulation of deep brain areas opens a wide range of therapeutic and research op-

tions. Optimization of stimulation parameters requires further investigation into mechanisms utilizing imaging and electrophysiological techniques.

S9.4
FROM OPTOGENETICS TO A NOVEL CLINICAL TREATMENT AGAINST COCAINE USE DISORDERS

Antonello Bonci
National Institute on Drug Abuse, Scientific Director 251 Bayview Blvd. Baltimore, Maryland, USA

The main goal of my talk will be to discuss the role of long-term plasticity at excitatory synapses in the limbic system in modulating the development and expression of cocaine-dependent behaviors, in order to produce novel therapeutic strategies that could reverse these long-term synaptic changes, and as a consequence, drug-dependent behaviors. During my lecture I will present published and unpublished results on the basic mechanisms that underlie cocaine-dependent plasticity, and the promising results from our clinical studies that were created based on our results obtained with optogenetics.

S10 Room: Reading Room
OXIDATIVE STRESS IMPLICATION IN NEUROPATHOLOGY

Chairs: Amira Zaky, EG and Nicola Mercuri, IT

S10.1
APE1 PARADOX IN NEUROPATHIC PAIN MANAGEMENT: NOVEL INSIGHT ON ITS ROLE AS A REGULATOR FOR MICRORNAS

Amira Zaky
Department of Biochemistry, Faculty of Science, Alexandria University, Moharram Bake, P.O Box 21511, Alexandria, Egypt and University of Bordeaux, CNRS, IINS-UMR5297, Bordeaux, France

Treatment of neuropathic pain remains difficult, in part, due to the weakness of the knowledge about the mechanisms underlying chronic pain pathogenesis. Chronic pain caused by peripheral nerve injury is associated with oxidative stress and global changes in gene expression in damaged neurons. Apurinic/apyrimidinic endonuclease 1 (APE1), a Redox Factor-1 (Ref-1), plays an essential role in regulating various cellular functions including MicroRNAs (miRNAs) expressions and/or processing. In the current study we investigated APE1 modulation and associated behavior changes in Complete Freund's adjuvant (CFA)-induced rats. In addition we tested the anti-inflammatory efficiency of the chemical compound E3330 as a selective APE1 redox activity inhibitor.

Herein we show that APE1 sub-cellular localization and expression are altered significantly in CFA-induced rats at day four. We observed more nuclear localization in the induced versus sham groups accompanied by around 30% reduction in APE1 mRNA level. On the contrary, an elevated cytosolic accumulation and normal expression are detected in the group co-injected with E3330. Furthermore, Co-injection of the selective APE1 redox inhibitor E3330 to CFA induction ameliorated significantly the induced inflammation and nociception as detected by Paw withdrawal Von Frey test. A key pain modulators mir-134 and mir-219-5p levels were also altered significantly in the different experimental groups in parallel to APE1 sub-cellular distribution as also confirmed by *in vitro* studies.

In conclusion, we show that APE1 expression and cytosolic versus nuclear localization are implicated in neuropathic pain sensitization. This study shed the light on the possible involvement of APE1 protein specific activity in the regulation of target micro-RNA levels. Further studies are required to elucidate the exact mechanism of APE1 in neuropathic condition.

S10.2
SELENOPROTEINS, OXIDATIVE STRESS AND PARKINSON'S DISEASE

Youssef Anouar
Normandy University of Rouen, Inserm U982, Lab. Neuronal and Neuroendocrine Differenciation and Communication, 76821 Mont-Saint-Aignan, France

Oxidative stress is central to the pathogenesis of Parkinson's disease (PD), but the mechanisms involved in the control of this stress in dopaminergic cells are not fully understood. There is increasing evidence that selenoproteins play a central role in the control of redox homeostasis and cell defence, but the precise contribution of members of this family of proteins during the course of neurodegenerative diseases is still elusive. We demonstrated that a novel selenoprotein named selenoprotein T (SelT) whose gene disruption is lethal during embryogenesis, exerts a potent oxidoreductase activity that is essential for the protection of dopaminergic neurons. In the SH-SY5Y cell model of dopaminergic neurons, both silencing and overexpression of SelT affected oxidative stress and cell survival. Treatment with PD-inducing neurotoxins such as 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) or rotenone triggered SelT expression in the nigrostriatal pathway of wild-type mice, but provoked rapid and severe parkinsonian-like motor defects in conditional brain SelT-deficient mice. This motor impairment was associated with marked oxidative stress and neurodegeneration, and decreased tyrosine hydroxylase activity and dopamine levels in the

nigrostriatal system. Finally, in PD patients, we report that SelT is tremendously increased in the caudate putamen tissue. These results reveal the activity of a novel selenoprotein enzyme that protects dopaminergic neurons against oxidative stress and prevents early and severe movement impairment in animal models of PD. Our findings indicate that selenoproteins such as SelT play a crucial role in the protection of dopaminergic neurons against oxidative stress and cell death, providing insight into the molecular underpinnings of this stress in PD.

S10.3
DOPAMINERGIC DYSREGULATION IN ANIMAL MODELS OF DEGENERATIVE DISEASES

Nicola Biagio Mercuri
Fondazione Santa Lucia IRCCS, Via del Fosso di Fiorano 64, 00143, Rome, Italy; Department of Systems Medicine, Via Montpellier 1, 00133, University of Tor Vergata, Rome, Italy

Lewy bodies and Lewy neurites are the principal features of the neurodegenerative processes of substantia nigra pars compacta (SNpc) dopaminergic neurons in Parkinson's disease (PD) and of other synucleinopathiesand are characterized by the presence of α-synuclein (α-syn).

There are Berlin-Druckrey rats carrying a spontaneous mutation in the 3′ untranslated region of α-syn mRNA (*m/m* rats) that display a widespread accumulation of α-syn in the mesencephalon, striatum and frontal cortex. In spite of this, there is only a slight reduction of SNpc and ventral tegmental area DAergic cells. The dopaminergic neurons of the *m/m* rats have a reduced Ih conductance and a reduced frequency of spontaneous excitatory synaptic currents but do not show clear-cut alterations of their physiological and pharmacological properties when recorded electrophysiologically at the somato-dendritic level, *in vitro* conditions. Contrariwise, *m/m* rats show a severe impairment of DA and glutamate release in the dorsolateral striatum, as revealed by amperometry (DA) and by electrophysiological recordings of glutamatergic synaptic events in striatal medium spiny neurons. These functional impairments are associated to a decreased expression of the DA transporter and VGluT1 proteins in the same area. Thus, α-syn overload in the mesencephalic region, striatum and frontal cortex alters the function of DAergic and glutamatergic terminals in the dorsal striatum of the *m/m* rats.

S11 Room: Marie Louise 1
NEUROCYTOSKELETON FUNCTIONS AND DYSFUNCTIONS IN NEURODEGENERATIVE AND PSYCHIATRIC DISEASES

Chairs: Annie Andrieux, FR and Marie Jo Mountin, FR

S11.1
CROSSTALK BETWEEN MICROTUBULAR PROTEINS MAPS AND +TIPS IN NEURONAL CELLS: POSSIBLE IMPLICATIONS IN NEURODEGENERATION

Laura C. Sayas
Centro de Biología Molecular "Severo Ochoa" (CSIC-UAM), Madrid, Spain; Centro de Investigación Biomédica en Red de Enfermedades Neurodegenerativas (CIBERNED), Madrid, Spain; INSERM U836, Grenoble Institut des Neurosciences, Grenoble, France

Microtubules (MTs) are key cytoskeletal elements that play important roles in neuronal polarity and differentiation, maintenance of neuronal architecture, intracellular transport of cargos and as scaffolds for signaling molecules. Hence, MT dynamics and stability require fine-tuned regulation, for the proper differentiation and functioning of neurons. Different types of proteins contribute to the regulation of the MT state, such as classical microtubule-associated proteins (MAPs), which bind along the microtubule lattice, and plus-end tracking proteins (+TIPs), which interact with the plus-ends of growing microtubules. We have recently shown a direct interplay between classical MAPs (e.g. tau) and End Binding Proteins (EBs), the core +TIPs, in neuronal cells1-5. This may contribute to the orchestrated regulation of MT dynamics/stability in neurons. Perturbations in the balance between dynamic and stable MTs have been observed in different neurodegenerative disorders such as Alzheimer's disease (AD) or other tauopathies. Based on this, we aim to address whether the dysregulation of the MAPs/+TIPs crosstalk might contribute to the progression of neurodegeneration.

S11.2
TUBULIN MODIFYING ENZYMES AND NEURODEGENERATION

Marie-Jo Moutin
Institut des Neurosciences de Grenoble, INSERM U836, CEA, Université Grenoble Alpes, 38042 Grenoble, France and Institut Curie, CNRS UMR3348, Orsay, France

Tubulin is subject to a range of post-translational modifications. Most of them generate chemical marks at the C-terminal tail of tubulin which project outside microtubules (MT) and select MT for specific cellular functions by regulating their interactions with proteins partners and influencing their stability/dynamics. Among these modifications is the cycle of tyrosine removal and

re-addition at the α-tubulin C-terminus, which involves a peptidase producing detyrosinated tubulin and TTL (tubulin tyrosine ligase) which re-adds the tyrosine. Another modification that generates a huge variety of C-terminal tail versions is the enzymatic polyglutamylation of both α- and β-subunits catalyzed by the families of tubulin tyrosine ligase-like proteins (TTLLs) and of reverse enzymes, the cytosolic carboxypeptidases (CCPs).

The right balance between stable and dynamics MT is crucial for neuronal life and survival (stability is essential to axon maintenance, dynamics to synaptic plasticity) and defects of both MT stability and dynamics are known to be involved in neurodegeneration.

We will present data demonstrating the crucial role of polyglutamylation in the pcd mice neurodegeneration. In these mice, tubulin over-glutamylation linked to CCP1 absence, is responsible for the degeneration, and the downregulation of TTLL1 (the tubulin specific neuronal polyglutamylase) in the cerebellum of young mice prevent Purkinje cells death and improve motor coordination.

We will present recent data regarding tyrosinated and non-tyrosinated tubulin variants in brain samples of Alzheimer patients as well as relationship tubulin tyrosination and dentritic spines' integrity in neuronal models related to Alzheimer conditions (neurons exposed to Aβ peptides).

Altogether we will document the fact that a correct amount/activity of tubulin modifying enzymes involved in the balance between stable and dynamic MT is crucial to neuron survival, and, as a consequence, that these enzymes might be pertinent targets to develop molecules able to slow down progression of neurodegenerative diseases.

S11.3
POSITIVE EFFECTS OF CYTOSKELETON-RELATED DRUGS ON COGNITIVE ABILITIES, IN AN ANIMAL MODEL OF PSYCHIATRIC DISEASES

Annie Andrieux
INSERM U1216, Grenoble Institute of Neuroscience, University Grenoble Alpes, France and CEA BIG-GPC, University Grenoble Alpes, France

During neurodevelopment, cytoskeletal elements, including actin filaments and microtubules, play major roles. Microtubules are highly dynamic structures involved in morphogenetic and plasticity events in neurons. For example, during neurodevelopment, microtubules contribute to axonal growth and spine formation; whereas in the adult brain they are involved in events related to synaptic plasticity. Several microtubule regulators have been identified which control their behavior in neurons, such as the microtubule associated protein, MAP6 (also called STOP), which plays a number of essential roles.

In mice, MAP6 deletion has dramatic effects on integrated brain functions. Thus, MAP6-null mice display multiple behavioral disorders including cognitive impairments associated with strong synaptic plasticity. Some of these alterations to integrated brains functions were shown to be related to brain connectivity defects and cellular abnormalities. These effects tie in well with the functions of MAP6, which significantly contributes to neuronal connectivity, axonal tract formation, semaphorin3E signaling, and also helps maintain dendritic spine integrity.

Also, the biological and behavioral disorders of MAP6/STOP-null mice are reminiscent of some of the symptoms observed in schizophrenia. In line with this similarity, both typical and atypical antipsychotic treatments have been shown to alleviate the synaptic and behavioral deficits in these mice. We will discuss the effects of cytoskeletal-related drugs (Epothilone D, Davunetide, LIM Kinase inhibitor) on MAP6-null mice in terms of cognitive abilities at behavioral and biological levels.

S11.4
MICROTUBULE DYSFUNCTION IN ANIMAL MODELS OF PARKINSON'S DISEASE

Graziella Cappelletti
Dept. Biosciences, Università degli Studi di Milano, Milano, Italy

Looking at the multiple hit hypotheses in Parkinson's diseases (PD), up to now a marginal attention has been dedicated to the microtubule (MT) system. Indeed, the concept that MT dysfunctions can participate in, and perhaps lead to, PD progression, has been supported by evidence coming from toxin-based and genetic experimental models of the pathology. In the context of studies on PD-induced neurotoxins, we previously investigated MTs in MPTP mouse model of the disease unravelling that MT alterations occur as an early event specifically associated to dopaminergic neuron degeneration and that their pharmacological stabilization may be a potential strategy for the management of experimental parkinsonism. In the context of studies on models of genetic PD, we are currently studying the impact of mutations in parkin and α-synuclein on function and dysfunction of the MT system. *Parkin* gene, whose mutations are responsible for the majority of the Autosomal Recessive Juvenile Parkinsonism, codes for an E3 ligase catalysing the addition of ubiquitin to target proteins included α- and α-tubulin. However, parkin interaction with tubulin and MTs has been poorly investig-

ated for a long time. Interestingly, the recent evidence that the destabilization of MTs occurs in fibroblasts and in induced-pluripotent stem cell (iPSC)-derived neurons from PD patients adds impulse to further investigation. Starting from parkin-silenced cells and primary midbrain neurons obtained from *Parkin* knockout mice, we performed live cell imaging to investigate MT dynamics and axonal transport. We observed that parkin absence induces a faster MT growth, thus making MT more dynamics, and triggers the impairment of mitochondria transport. Interestingly, the treatment with the MT stabilizer Taxol rescues the mitochondria transport defects. Next, we moved to Parkin mouse models that include Parkin knockout, *Parkin* heterozygous and *Parkin*-Q311X mice. In differently aged mice, we analysed tubulin post-translational modifications that are usually used as marker of MT with different stability, being tyrosinated the most dynamic pool and detyrosinated or acetylated more stable subsets. We found that the unbalancing in tubulin modification pattern occurs very early, accumulates over time, and precedes defects in axonal transport.

Moving to α-synuclein, the first protein associated to familial PD, it is a given that the previously published biochemical studies of α-synuclein/tubulin interaction are contradictory. We have very recently reported that wild-type α-synuclein induces MT nucleation and governs multiple steps of MT dynamics both in purified systems and in neuronal cells and, notably, that PD-linked mutations impact on this property of α-synuclein and trigger MT aggregation. We are currently focusing on the impact of α-synuclein on MT cytoskeleton in murine models.

Collectively, our data converge on the view that MT dysfunction occurs very early in the onset of neuronal damage in PD models and, indeed, strongly suggest that MTs could represent a crucial culprit in causing this disabling disease and a reliable target for neuroprotection.

S11.5
ACTIN CYTOSKELETON DISRUPTION AS A NOVEL PLAYER IN THE PATHOGENESIS OF FAMILIAL AMYLOID POLYNEUROPATHY

Marcia A. Liz
Neurodegeneration Group, IBMC - Instituto de Biologia Molecular e Celular, Universidade do Porto; Instituto de Investigação e Inovação em Saúde, Universidade do Porto, Portugal

Familial Amyloid Polyneuropathy (FAP) is a neurodegenerative disease characterized by deposition of amyloid fibrils of mutated transthyretin (TTR) in the peripheral nervous system, leading to a dying-back axon degeneration. Abnormalities in cytoskeletal organization are a common feature of many neurodegenerative disorders. In this work we investigated the hypothesis that cytoskeleton damage occurs downstream of TTR deposition. In primary cultures of mouse dorsal root ganglia (DRG) neurons, a relevant cell type for FAP studies as mutant TTR accumulates close to the DRG, axons treated with TTR oligomers presented a marked reduction of the growth cone area, with disruption of the typical morphology of the growth cone which lacked the lamellipodial actin structures. Additionally, using a FAP *Drosophila* model in which the amyloidogenic mutant TTR Val30Met is expressed in the photoreceptor cells resulting in roughening of the eye, we observed decreased axonal projection of photoreceptor neurons that presented more compact growth cones lacking the spread distribution of filopodia and lamellipodia actin structures. A genetic screen was subsequently performed by crossing the TTR Val30Met flies with readily available fly lines for the knockdown or overexpression of candidate genes whose function is associated with cytoskeleton dynamics. In this screen we determined that the Rho GTPase family-the major regulator of actin dynamics modulates TTR-induced rough eye phenotype. Our results suggest a disruption of the actin cytoskeleton upon mutant TTR deposition. Future work will dissect the cascade of events that underlie alterations in axonal cytoskeleton dynamics induced by TTR and validate actin cytoskeleton damage in a FAP mouse model.

S12 Room: Marie Louise 2
GENETIC AND EPIGENETIC REGULATION OF STRESS SENSITIVITY FROM MOUSE TO MAN: RELEVANCE TO STRESS-RELATED DISORDERS

Chair: Nicolas Singewald, AT

S12.1
GENETIC AND EPIGENETIC FACTORS THAT MODULATE STRESS-INDUCED NEURO- AND GLIO-PLASTICITY: RELEVANCE TO DEPRESSION

Luisa Alexandra Meireles Pinto
Life and Health Sciences Research Institute (ICVS), School of Health Sciences, University of Minho, Braga, Portugal

A fundamental hallmark of modern neuroscience lies on the realization that, similarly to what happens in other tissues of our body, the adult mammalian central nervous system is endowed with considerable regenerative potential. This regenerative capacity is reflected in cellular processes defining the so-called neural plas-

ticity, which include rapid and dynamic axo-dendritic remodelling of post-mitotic cells, as well as the ability of restricted brain regions to persistently generate new neuronal and glial cells, a process known as neuro- and gliogenesis, respectively. Together, neural plasticity confers to the mammalian brain a wide adaptive capability to redefine neuro-glial circuits, in response to every-day-life experiences and challenges.

Post-natal neuroplasticity is largely driven by the transduction of environmental stimuli into essential neuroadaptations. Neuroplastic maladaptations often result in the manifestation of pathological traits, from which depressive behavior is a paradigmatic example. We are investigating the pathological basis of both physiological and behavioral impairments and their potential epigenetic molecular determinants. It is also our aim to study how depression and antidepressant drugs can modulate epigenetic patterns in key limbic areas and how this impacts in the transduction of their effects.

In this talk I will focus on the mechanistic link between neuro- and glio-plasticity and depression, taking into account the dynamic spatio-temporal events that define plasticity and the dichotomy between dorsal and ventral hippocampus. The central question is: What are the genetic and epigenetic factors that modulate neuro- and glio-plasticity and impact on brain neuronal networks so as to elicit a depressive state? We intend to dissect the molecular cascade leading to neuron-glia/behavioral dysfunction to gain insights into the underpinnings of susceptibility and resilience to depression.

S12.2
ADULT HIPPOCAMPAL NEUROGENESIS AND VULNERABILITY TO ACUTE AND TO CHRONIC STRESSORS

Catherine Belzung
Inserm 930 Imaging and Brain, University of Tours & Institut Universitaire de France UFR Sciences et Techniques, Parc Grandmont, F-37200 Tours, France

Stress is a key player in the aetiology of most psychiatric disorders. Particularly, acute traumatic stress is inducing post-traumatic stress disorder (PTSD) is some individuals, others being resilient. The same is observed in major depression as chronic stress triggers episodes of major depression, only in some vulnerable individuals. The hippocampus is a brain structure involved in the sensitivity to stressors. For example, structural neuroimaging studies have shown that the volume of the hippocampus is involved in the development of PTSD after a trauma. Adult newborn neurons are generated through life in the dentate gyrus of the hippocampus and therefore one can hypothesize that their density/functional properties may be involved in the sensitivity to develop PTSD or depressive-like symptoms after traumatic or chronic stress, respectively. We will present data obtained in mice having an increase in adult-generated neurogenesis (Ibax mice) and confronted, at different time-points after induction of neurogenesis, either to acute traumatic stress (an electric footshock) or to an unpredictable chronic mild stress. We show that adult newborn neurogenesis can protect against the detrimental effects of stress and that this depends upon the timing of the increase in neurogenesis. Underlying neurobiological mechanisms have been investigated as well, suggesting that adult neurogenesis can impact the activity of extra-hippocampal brain areas.

S12.3
(EPI)GENETIC REGULATION OF STRESS AND FEAR SENSITIVITY IN MOUSE MODELS OF ANXIETY

Nicolas Singewald
Department of Pharmacology and Toxicology, Institute of Pharmacy and CMBI, Leopold-Franzens-University of Innsbruck, Innsbruck, Austria

Anxiety and trauma-related disorders are the most common of all mental disorders, affecting 14% of Europeans each year, with a lifetime prevalence of 28% in developed countries. The development of improved treatment for these highly prevalent stress-related pathologies is one particularly important challenge of the 21st century. Individual differences in fear and fear extinction learning are proposed to modulate vulnerability to develop and maintain anxiety and trauma-related disorders. One promising avenue for drug development in this field is to improve the efficacy of exposure-based therapy, an important treatment option for these disorders. However, the mechanisms underlying extinction, as well as possible ways of enhancing magnitude and duration of therapeutic effects are incompletely understood. Epigenetic mechanisms are known to play a role in neuronal plasticity, including the formation of long-lasting extinction memories, the main mechanism underlying successful exposure-based therapy. To study the role of epigenetic mechanisms in fear extinction failure and its rescue, we used 129/SvlmJ (S1) mice, which display a profound deficit in fear extinction learning. Comparing extinction-impaired S1 to normally extinguishing 129S6 or C57Bl/6 mice, we revealed a number of aberrantly expressed histone marks, chromatin remodeling factors and miRNAs in fear extinction learning-associated brain areas of S1 mice. Rescue of impaired fear extinction in S1 mice was associated with an altered expression of a number of learning and memory-related coding genes, increased histone acetylation in some of these genes and changes in miRNA regulation including normalisation of

aberrantly expressed miRNAs. Among these, we showed the increased expression of miR-144 to be specific for extinction but not fear learning and identified a number of extinction-relevant target genes of this miRNA, which regulate MAPK/ERK and PI3/AKT signaling cascades. Detailed anatomical analysis revealed robust expression of miR-144 in the basolateral amygdala. Finally, we prove causal relationships and show examples that targeting these epigenetic mechanisms indeed can normalize deficits in the formation of enduring fear extinction memories. These findings may pave new ways for the development of improved treatment strategies in anxiety and trauma-related disorders.

S12.4
GENETIC MODERATION OF STRESS SYSTEMS INFLUENCING FEAR EXTINCTION

Andrew Holmes
Laboratory of Behavioral and Genomic Neuroscience National Institute on Alcohol Abuse and Alcoholism Rockville, MD, USA

Recent years have seen advances in our understanding of the neural circuits associated with trauma-related disorders, and the development of relevant assays for these behaviors in rodents. Although inherited factors are known to influence individual differences in risk for these disorders, it has been difficult to identify specific genes that moderate circuit functions to affect trauma-related behaviors. Here, we exploited robust inbred mouse strain differences in Pavlovian fear extinction to uncover quantitative trait loci (QTL) associated with this trait. We found these strain differences to be resistant to developmental cross-fostering and associated with anatomical variation in basolateral amygdala (BLA) perineuronal nets, which are developmentally implicated in extinction. Next, by profiling extinction-driven BLA expression of QTL-linked genes, we nominated *Ppid* (peptidylprolyl isomerase D, a member of the tetratricopeptide repeat (TPR) protein family) as an extinction-related candidate gene. We then showed that Ppid was enriched in excitatory and inhibitory BLA neuronal populations, but at lower levels in the extinction-impaired mouse strain. Using a virus-based approach to directly regulate Ppid function, we demonstrated that downregulating BLA-Ppid impaired extinction, while upregulating BLA-Ppid facilitated extinction and altered *in vivo* neuronal extinction-encoding. Next, we showed that Ppid colocalized with the glucocorticoid receptor (GR) in BLA neurons and found that the extinction-facilitating effects of *Ppid* upregulation were blocked by a GR antagonist. Collectively, our results identify *Ppid* as a novel gene involved in regulating extinction via functional actions in the BLA, which could potentially have implications for understanding the genetic and pathophysiological mechanisms underlying risk for trauma-related disorders.

S12.5
MICROBIAL GENES, BRAIN AND BEHAVIOUR: REGULATION OF STRESS SUSCEPTIBILITY BY THE MICROBIOME

John F. Cryan
Dept. Anatomy & Neuroscience, University College Cork, Cork, Ireland

There is a growing emphasis on the relationship between the complexity and diversity of the microorganisms that inhabit our gut (human gastrointestinal microbiota) and health/disease, including brain health and stress susceptibility. The microbiota-gut-brain axis is a dynamic matrix of tissues and organs including the brain, glands, gut, immune cells and gastrointestinal microbiota that communicate in a complex multidirectional manner to maintain homeostasis.

The routes of communication between the microbiota and brain are being unravelled and include the vagus nerve, gut hormone signalling, the immune system, tryptophan metabolism or by way of microbial metabolites such as short chain fatty acids. The importance of early life gut microbiota in shaping future health outcomes is also emerging.

Disturbances of this composition by way of antibiotic exposure, lack of breastfeeding, infection, stress and the environmental influences coupled with the influence of host genetics can result in long-term effects on physiology and behaviour, at least in animal models. It is also worth noting that mode of delivery at birth influences microbiota composition with those born by Caesarean section having a distinctly different microbiota in early life to those born per vaginum. At the other extreme of life, ageing is associated with a narrowing in microbial diversity and healthy ageing correlates with a diverse microbiome.

Recently, the gut microbiota has been implicated in gating how the host deals with stress. It is plausible that stress-related disorders might be treated in the future by targeting the microbiota either by microbiota transplantation, antibiotics or psychobiotics (prebiotics or probiotics).

S13 Room: Ballroom
5-HT$_{2C}$ RECEPTORS: WIDESPREAD CONTROL OF NEUROBIOLOGICAL NETWORKS: II

Chairs: Giuseppe Di Giovanni, MT and Umberto Spampinato, FR

S13.1
5-HT$_2$ RECEPTORS AND MEMORY

Alfredo Meneses
Departmento de Farmacobiología, CINVESTAV México

Serotonin (5-hydroxytryptamine, 5-HT) systems have been well-characterized in mammal species, including multiple receptors (5-HT$_{1A/1B/1D}$, 5-HT$_{2A/2B/2C}$, 5-HT$_3$, 5-HT$_4$, 5-HT$_5$, 5-HT$_6$, and 5-HT$_7$ receptors), transporter (SERT) and volume transmission. These serotonergic markers have been involved in memory and dementias. For instance, 5-HT$_{2A/2B/2C}$ receptors have been useful detecting memory changes and drug effects. Indeed, decreased 5-HT2A receptor expression is observed in patients with MCI. In addition, an association between 5-HT$_{2A}$ receptor expression and memory formation has been reported. Certainly decreased 5-HT$_{2A}$ (and 5-HT$_{1A}$) receptors expression in AD patients has been observed. Although an association between 5-HT$_{2A}$ receptor polymorphism (his452tyr) and memory performances in AD has been proposed; no differences in verbal memory were identified. However, individual differences exist in impulsive action and cortical 5-HT$_{2A}$ receptor, physiological modulation of prefrontal cortex efficiency during working memory, and response to antipsychotics. As well as 5-HT$_{2A}$ receptor and individual variation are observed in spatial-discrimination serial reversal learning, behavioral inflexibility and other forms of compulsive behavior. It is unclear why in some cortical regions either stimulation or blockade of 5-HT$_{2A}$ receptor produces antiamnesic effects. Moreover, it has been investigated mechanisms of action of psychoactive drugs, which modestly benefit cognitive performance in fragile X patients (the most common form of inherited mental retardation). 5-HT$_{2B}$ receptor activation or 5-HT$_{2A}$ receptor inhibition moderately enhanced Ras-PI3K/PKB signaling input, GluA1-dependent synaptic plasticity, and learning. In addition, genetic deletion of 5-HT$_{2B}$ receptor elicits deficits in sensorimotor gating, selective attention, social interaction, fear conditioning and novel object recognition. Certainly, 5-HT$_{2A}$ receptor stimulation produces multi-faceted modulation on memory and cognition, including memory consolidation and may represent a new neuropharmacological target for the treatment of memory impairments. Among other actions, 5-HT$_{2C}$ receptor blockade produces antiamnesic effect, hippocampal neuronal cell survival, neurogenesis, and neuroprotective effect in hippocampus and frontal cortex in a model of epilepsy. However, the 5-HT$_{2C}$ inverse agonist mianserin impaired operant memory. Moreover, use of microwaves impact health including cognitive function in which neurotransmitter system plays an important role; particularly, exposure to microwave impairs spatial memory and produces disturbance of hippocampal and cortical expression of 5-HT$_{1A}$ and 5-HT$_{2C}$ receptors. In this context, we should bear in mind the inverse agonism at 5-HT$_{2A}$ and 5-HT$_{2C}$ receptors.

S13.2
5-HT$_{2C}$ RECEPTORS ARE A KEY PHARMACOLOGICAL TARGET TO CONTROL DA NEURON ACTIVITY AND DA-DEPENDENT BEHAVIORS: FOCUS ON COCAINE

Umberto Spampinato
Bordeaux University, Bordeaux, France and Inserm U1215, Neurocentre Magendie, Physiopathology of Addiction Group, Bordeaux, France

The serotonin$_{2C}$ receptor (5-HT$_{2C}$R), in keeping with its ability to control the mesoaccumbens dopamine (DA) pathway, plays a key role in mediating the behavioral and neurochemical effects of drugs of abuse. Studies assessing the influence of 5-HT$_{2C}$R agonists on cocaine-induced responses have suggested that 5-HT$_{2C}$Rs can modulate mesoaccumbens DA pathway activity independently of accumbal DA release, thereby controlling DA transmission in the nucleus accumbens (NAc). In the present study, we assessed this hypothesis by studying the influence of the 5-HT$_{2C}$R agonist Ro 60-0175 on cocaine-induced behavioral, neurochemical and molecular responses. The intraperitoneal (i.p.) administration of 1 mg/kg Ro 60-0175 inhibited hyperlocomotion induced by cocaine (15 mg/kg, i.p.), had no effect on cocaine-induced DA outflow in the shell and increased it in the core subregion of the NAc. Also, Ro 60-0175 inhibited the late-onset locomotion induced by the subcutaneous administration of the DA-D2R agonist quinpirole (0.5 mg/kg), as well as cocaine-induced increase in c-Fos immunoreactivity in NAc subregions. Finally, Ro 60-0175 inhibited cocaine-induced phosphorylation of the DA and c-AMP regulated phosphoprotein of Mr 32kDa (DARPP-32) at threonine residues in the NAc core, this effect being reversed by the selective 5-HT$_{2C}$R antagonist SB 242084 (0.5 mg/kg, i.p.).

Altogether, these findings demonstrate that 5-HT$_{2C}$Rs modulates mesoaccumbens DA pathway activity at post-synaptic level, by specifically controlling DA signaling in the NAc core subregion. This interaction, in keeping with the tight relationship between locomotor activity and NAc DA function, could participate in the inhibitory control of cocaine-induced locomotor activity. Altogether, the obtained results afford additional knowledge into the prominent role of the 5-HT$_{2C}$R into the regulatory neurochemistry of mesoaccumbens DA functions, and provide new information allowing a better understanding of the mechanisms underlying the 5-HT$_{2C}$R-dependent control of cocaine-induced responses.

S13.3
5-HT_{2A} AND 5-HT_{2C} RECEPTORS EXPRESSION PROFILE IN YOUNG AND ADULT GAERS, NEC AND WISTAR RATS

Cristiano Bombardi
University of Bologna, Department of Veterinary Medical Sciences, Bologna, Italy

Alterations of the serotonin (5-HT) activity is associated with seizure generation and neuronal network activity. Most evidence suggests that 5-HT_{2A} and 5-HT_{2C} receptor subtypes (R) are involved in absence seizures (ASs) generation. Accordingly, the expression pattern of immunohistochemical distribution of the 5-HT_{2A} and 5-HT_{2C}Rs is different comparing adult (P90) and young (P25) non-epileptic control (NEC) and Wistar rats with Genetic Absence Epilepsy Rats from Strasbourg (GAERS).

At P90, the 5-HT_{2A}R immunoreactivity is associated with somatodendritic profiles and neuropil throughout the RTN (reticular thalamuc nucleus), VB (ventrobasal thalamus), DLGN (dorsal lateral geniculate nucleus), M1 (primary motor cortex), and S1po (pero-oral region of the primary somatosensory cortex). A high density of immunopositive somata is present in the M1, S1po and, to some extent, in the thalamus (RTN, VB, DLGN). The neuropil staining, consisting of diffuse staining and dendrites, varied in different area. The diffuse neuropilar labeling can not be associated with any specific neuronal elements. The highest intensity of neuropil labelling is present in DLGN, RN, M1, and S1po. The image area covered by 5-HT_{2A} immunostaining is high in RTN, M1 and S1po. In the RTN, VB, DLGN, M1, and S1po 5-HT_{2C}R immunolabeling is found in cell bodies and neuropil. Every examined area contain a high number of immunostained somata. The neuropil immunoreacticvity, consisting only of diffuse staining, is not associated with any identifiable neuronal structure. The density of diffuse neuropilar staining is evident in every area. The image area covered by 5-HT2C immunostaining is high in M1 and S1po. Generally, the 5-HT_{2A}R - and 5-HT_{2C}R - immunoreactivity patterns is similar in the RTN, VB, DLGN, M1, and S1po of NEC and Wistar rats. On the contrary, the 5-HT_{2A}R -immunoreactivity is more evident in GAERS than in NEC rats. The 5-HT_{2C}R -immunoreactivity is very similar in NEC, Wistar and GAERS rats. However, the intensity of the diffuse neuropil staining is particularly high in NEC rats.

At P25, the 5-HT_{2A}R- and 5-HT_{2C}R-immunoreactivity patterns is similar in NEC and Wistar rats. The immunoreactivity for the 5-HT_{2A}R is located in somata and neuropil. In the NRT, the density of immunopositive neurons is significantly higher in NEC than in GAERS. The density of 5-HT_{2A}R-immunoreactive neurons in the VB shows no significant differences between NEC and GAERS rats. In the NRT and VB, the percentage of the image covered by 5-HT_{2A}R immunoreactivity, as well as the neuropilar immunostaining, are similar in NEC and GAERS rats. In the NRT and VB, the 5-HT_{2C}R immunoreactivity is found to be associated with somata and diffuse neuropil in NEC, Wistar and GAERS. Generally, the 5-HT_{2C}R immunoreactivity patterns are similar in NRT and VB of different rats. However, the diffuse neuropil immunostaining of the VB is more evident in GAERS than in NEC rats.

In conclusion, at different ages, the dysregulation of the 5-HT_{2A}R - and 5-HT_{2C}R might be involved in the pathogenesis of the ASs in GAERS or, alternatively, may be a consequence of the seizures.

S14 Room: Clermont Suite
THE SICK BRAIN

Chair: Ana María Sánchez-Pérez, ES

S14.1
G-PROTEIN COUPLED RECEPTOR HETERODIMERS: POWERFUL DRUG TARGETS FOR THE TREATMENT OF DISEASE

Peter McCormick
University of Surrey, UK

G-protein coupled receptors continue to represent a third of all new drug targets. Recent structural breakthroughs on these receptors have demonstrated how important cellular and physiological context are to understanding how these receptors can be exploited for therapeutic purposes. Our work illustrates these points by showing how G-protein coupled receptors can form higher order complexes in select brain regions. These complexes can alter both receptor function and neuronal response to neurotransmitters with profound physiological changes in both healthy and disease states.

S14.2
A RANDOMISED CONTROLLED TRIAL OF DEEP BRAIN STIMULATION IN SEVERE REFRACTORY OBSESSIVE COMPULSIVE DISORDER

Ludvic Zrinzo
UCL Institute of Neurology, London

A significant minority of patients with Obsessive Compulsive Disorder (OCD) remain severely affected despite conventional treatment. We present the clinical results of a double-blind randomised crossover pilot trial of deep brain stimulation (DBS) for OCD. Six patients with severe refractory OCD were recruited. Inclu-

sion criteria were: symptoms refractory to $\geqslant 2$ selective serotonin reuptake inhibitors for $\geqslant 12$ weeks at optimal doses, $\geqslant 2$ trials of cognitive behavioural therapy (CBT) (> 10 hours), inpatient treatment; $\geqslant 10$ years' illness duration; $\geqslant 2$ years of unremitting symptoms; $\geqslant 32$ on the Yale-Brown Obsessive Compulsive Scale (YBOCS).

Bilateral anteromedial subthalamic nucleus (amSTN) and bilateral ventral capsule/ventral striatum (VC/VS) DBS leads were implanted in each patient using an MRI-guided & MRI-verified approach. Patients were randomised to amSTN or VC/VS stimulation. After 3 months, the stimulation site was switched for 3 month, then both sites were stimulated. for 3 months. Then, patients received open label DBS optimisation and CBT. Patients and psychiatrists were blinded to stimulation site during the randomisation phase. YBOCS and global assessment of function (GAF) scores were performed at key time points.

There were no surgical complications. YBOCS improved from baseline by a mean of 45% with amSTN DBS, 53% with VC/VS DBS and 61% with DBS at both sites. Following open label DBS and CBT, mean YBOCS reduction was 74%, 3 patients were in remission (YBOCS < 8), all patients were "responders" (defined as YBOCS decrease of $> 35\%$). GAF scores improved from 22 to 72.

DBS is efficient at either site with improvement in OCD symptoms that is also accompanied by improvements in quality of life scores. However, it requires close surgical and psychiatric follow up. DBS is a lifelong therapy that is safe and can be effective in well selected patients with severe refractory OCD.

S14.3
HIGH FAT DIET INDUCED NEUROINFLAMMATION AND COGNITIVE IMPAIRMENT CAN BE RESTORED BY FITOHORMONE ABSCISIC ACID TREAMENT

Ana María Sánchez-Pérez
Facultad de CC de la Salud, Universidad de Jaume I, Castellón de la Plana, (Castellón), Spain

The abscisic acid (ABA) is the main phytohormone involved in abiotic stress responses. However, ABA is not a molecule exclusive from plants but it can be found in many other organisms including bacteria, fungi and animals. Interestingly, it can be synthesized and secreted by a variety of human cells. Recent studies suggest a role of ABA regulating immune response and insulin action. Many neurological diseases have an inflammatory etiology and insulin resistance is a key factor in Alzheimer disease. Taking these data together we decided to ascertain if ABA has a protective effect in neuroinflammation, and brain insulin metabolism. We chose a model of neuroinflammation that involved feeding the animals with a High Fat Diet (HFD), this model induces glucose resistance and an increase of proinflammatory markers in peripheral tissues. Experimental groups included, HDF alone; HFD with ABA; and control diet with and without ABA. ABA was administered in the drinking water. We confirmed that, in our model, ABA restores glucose tolerance in HFD rats, to control levels. Behavior paradigms showed that HFD impairs lightly but significantly animal memory in T-maze but not in novel object recognition. ABA treatment is capable of reestablish the cognitive performance of HFD fed animals to control levels. We measured ABA concentration in blood and brain, confirming previous studies showing that ABA can cross the blood brain barrier. Moreover, we found that ABA administration in drinking water can curtail microglia increase induced by HFD. In addition, we measured several inflammatory markers in hypothalamus and hippocampus. We found that TNFα increased in hypothalamus of HFD but not in HFD-ABA groups. Interestingly, other reported neuroinflamamtoy markers analyzed did not change with HFD, these results will be discussed. Taking this results together, we propose that ABA might be a new therapeutic molecule to improve cognitive and metabolic processes associated to neuroinflammatory conditions.

S14.4
NEUROBEHAVIOURAL EFFECTS OF DEVELOPMENTAL TOXICITY OF HEAVY METALS

Mohamed Najimi
Biological Engineering Laboratory, Sultan Moulay Slimane University, Beni Mellal, Morocco

Human beings are exposed to complex mixtures of toxic compounds via food. This concerns principally pesticides and heavy metals. If in adults, low doses of these compounds are harmful, it could not be the same for a critical period like development. Indeed, these compounds are able to cross the placental barrier during pregnancy and to be present in milk during lactation and to interact directly or indirectly with metabolic pathways and hormonal synthesis involved in the development and the growth of many organs as well as their functional maturation. The mammalian brain is very sensitive to any change in metabolism during pre- and postnatal periods. To what extent heavy metals present in industrial « rejets » and/or pesticides, given at pregnancy and suckling pups, are responsible for brain damages at structural and/or functional level, this is the main objective of the present study.The experimental procedures consist of giving contaminated drinking water (by inorganic mercury) to pregnant rat females during the entire period of gestation and during the postnatal periods. The analysis of results shows that com-

pared to controls, rodent pups from intoxicated group are characterized by the presence of differences in some physical parameters namely an increase in the body weight, the size of the tail and a general delay in physical development. Concerning behavior, it is noteworthy that some parameters have been affected negatively such as motor orientation and coordination. This is well evidenced in negative geotaxis test (principally at postnatal PN 9 and PN 13), surface righting reflex (PN 5), Cliff avoidance (PN 6), and swimming development. At the adult stage, biochemical assays reported on an alteration of acetylcholinesterase activity and essential heavy metals distribution in many brain structures. These results indicate clearly that the developing brain is especially vulnerable to heavy metals even taken in low doses and that this vulnerability could have consequences in adulthood too.

S15 Room: Reading Room NOVEL PSYCHOACTIVE SUBSTANCES: BEHAVIOURAL, NEUROCHEMICAL, MOLECULAR EFFECTS AND UNDERLYING MECHANISMS

Chair: Maria Antonietta De Luca, IT

S15.1 NEUROPHARMACOLOGY OF NEWLY-EMERGING SYNTHETIC STIMULANTS: CATHINONES AND BEYOND

Michael H. Baumann
Designer Drug Research Unit, IRP, NIDA, NIH. Baltimore MD, USA 21224

Novel psychoactive substances (NPS) are synthetic alternatives to traditional drugs of abuse that are designed to circumvent existing drug control laws. NPS with stimulant-like properties exert their effects by targeting plasma membrane transporter proteins responsible for the uptake of dopamine, norepinephrine and serotonin (i.e., SLC6 monoamine transporter family). Stimulant drugs interact with transporter proteins as either inhibitors or substrates, and both types of drugs produce elevations in extracellular monoamine transmitters in the nervous system. In general, drugs which interact selectively with dopamine transporters display powerful rewarding effects whereas those which interact with serotonin transporters do not. The chemical structures of most stimulant-like NPS are based on the structure of cathinone, the beta-keto analogue of amphetamine. Cathinone-related NPS include mephedrone, methylone and their various analogues. More recently, a number of non-cathinone templates have been utilized to develop NPS, including indoleamines, benzofurans, and even prescribed medications like methylphenidate. In this presentation, we examine the interaction of stimulant-like NPS with monoamine transporters, along with their resulting neurochemical and behavioural effects. Specifically, data will be presented from *in vitro* transporter assays and *in vivo* paradigms such as microdialysis and locomotor stimulation.

S15.2 EFFECT OF THE KETAMINE-LIKE COMPOUND METHOXETAMINE ON BRAIN REWARD PROCESSING AND EMOTIONAL STATES

Liana Fattore
Institute of Neuroscience-Cagliari, National Research Council-Italy

Among the novel psychoactive substances, methoxetamine (MXE) is a ketamine analog that is emerging at an unprecedented rate on the Internet. Although perceived as safe by users, it induces severe adverse effects and intoxication cases has been described worldwide. To shed light on its pharmacological effects and potential underlying mechanisms, we performed a series of studies aimed to evaluate its effects on behavior, mood and reward in rats. The acute effects of MXE (0.5–5.0 mg/kg i.p.) were evaluated in different behavioral test, while its discriminative stimulus and positive reinforcing properties (MXE 0.125–0.500 i.v.) were assessed by using, respectively, the drug discrimination (DD) and the self-administration (SA) substitution chronic protocols. Neurochemical and electrophysiological experiments investigated its effect on the mesolimbic dopaminergic transmission, while a molecular study verified whether the observed behavioral responses correlated to rapid neuroadaptive molecular changes such as protein translation.

Data showed that at low and intermediate doses (0.5 and 1.0 mg/kg) MXE induces anxious and/or obsessive-compulsive traits (marble burying test), increases sociability (social interaction test) but does not induce spatial anxiety (elevated plus maze test) in rats. At the highest dose tested (5 mg/kg), MXE induces transient hypomotility and analgesia in the tail flick and hot plate test, and significantly reduces the time spent in immobility and climbing while increasing swimming activity in the forced swim test, suggesting an antidepressant effect. Moreover, MXE fully substitutes for ketamine interoceptive stimulus in the DD paradigm (showing to possess discriminative stimulus similar to ketamine) and substitutes for ketamine in a SA substitution study (showing to share common reinforcing properties with ketamine). MXE also stimulates meso-accumbal neurotransmission by activating VTA dopamine neurons and increasing dopamine level in the nucleus accumbens shell. Finally, immunohistochemistry study showed that

behaviorally active doses of MXE (1 and 5 mg/kg) increase the expression of phosphorylated ribosomal protein S6 (rpS6P) in the rat medial prefrontal cortex and hippocampus.

Altogether, our results indicate that MXE may differentially affect behavior and emotional states in rats depending on the dose tested, and that it activates the mesolimbic dopamine transmission which likely underlies its widespread use and abuse potential. As recently reported for ketamine, the increased expression of rpS6P protein in MXE-treated animals provides a potential correlate of rapid neuroadaptive changes induced by MXE.

S15.3
KETAMINE-INDUCED NEUROPLASTICITY AFTER ACUTE VS. CHRONIC SELF-ADMINISTRATION IN RATS: HOW TO KEEP APART "DR JEKYLL FROM MR HYDE"

Chiamulera Cristiano
Neuropsychopharmacology Lab, Section Pharmacology, Dept. Diagnostic & Public Health, Univ Verona, Verona

Ketamine is used as an anaesthetic but it is also abused with a high risk of psychotic effects. Moreover, ketamine was recently proposed as a treatment for major depression. The current debate is how to balance the risk/benefits of ketamine and ketamine-like drugs under development as antidepressant.

We investigated ketamine effects when given as an acute intravenous infusion (the proposed antidepressant regimen) or under an addictive regimen (chronic selfadministration; S/A) in rats. In a first set of experiments, we studied the pattern of neuroadaptive changes by measuring Zif-268 expression in key brain areas for addiction and depression, i.e., prefrontal cortex (PFC), ventral striatum (VS), hippocampus (H), and basolateral amygdala (BL). Then, we assessed BDNF release and downstream signalling in VS and hippocampus (H). Secondly, we focused on ketamine S/A effects on the expression of glutamatergic synapse components in medial PFC and H, two brain regions involved in addiction as well as in schizophrenia-related disorders.S/A, but not acute, group exhibited increased Zif-268, suggesting chronic ketamine-induced neuroadaptation. BDNF levels in H were increased in acute group (similarly to other antidepressants), whereas S/A group showed a decrease. In VS, BDNF decreased in both groups. In mPFC, ketamine S/A reduced expression of NMDA GluN1, GluN2A and GluN2B, whereas AMPA GluA1 and GluA2 were reduced in H only. However, NMDA and AMPA scaffolding proteins PSD-95 and SAP102 were both reduced in mPFC. Interestingly, metabotropic mGluR5 was also down-regulated in both areas.These findings show a regionally distinct modulation of BDNF that suggests dissociation between acute vs. S/A ketamine effects. Ketamine S/A induced overall reduction of post-synaptic glutamate receptors and impaired glutamate synapse homeostasis. This pattern of molecular effects may provide a reference model for studying either the therapeutic or abuse properties of ketamine-like drugs.

S15.4
POTENTIAL CLINICAL USE OF SOME LEGAL HIGHS

Colin Davidson
Pharmacy & Biomedical Sciences University of Central Lancashire Preston PR1 2HE England, UK

There have been a number of popular NPS with stimulant properties. The best known is mephedrone and this drug has been much researched over the last few years. There are a number of other stimulant-like NPS that have been popular for a few months or 1–2 years and these drugs have hardly been studied. Amongst these NPS are 'Benzofury' (5-APB, 5-MAPB), 'Ivory wave' (desoxypipradrol, 2DPMP), some methylphenidate-like drugs e.g. ethylphenidate and 3,4-CTMP and others.

We have investigated these drugs using a variety of techniques including fast-cyclic voltammetry (FCV) in rodent brain slices, radioligand binding and molecular modelling of interactions of these drugs at the dopamine transporter (DAT). FCV allows us to measure dopamine, noradrenaline or 5-HT efflux and uptake on a sub-second timescale. It can also differentiate between changes in phasic and tonic transmitter efflux as one might see with cocaine (increased phasic efflux) versus amphetamine (increased tonic efflux through reverse transport at the DAT). The radioligand binding studies have shown us the main targets of these drugs by measuring displacement of RTI121 (DAT), ketanserin (5-HT_{2A}), MK801 (NMDA) etc. The molecular modelling studies can be used to tease out mechanisms underlying the neurochemical effects.

Our data reveal that these stimulant-like NPS have varied pharmacologies with some resembling typical DAT inhibitors but with greater effects on dopamine efflux than cocaine (e.g. desoxipipradrol) while others can cause reverse transport of dopamine (5-APB, 5-MAPB, mephedrone) or might have both stimulant and hallucinogenic effects (5-APB).

These data will be discussed with reference to the potential dangers of taking these drugs.

S15.5
THIRD GENERATION SYNTHETIC CANNABINOIDS

Gaetano Di Chiara
Department of Biomedical Sciences, University of Cagliari, Italy

Herbal mixtures containing synthetic cannabinoid (SC) receptor agonists, used as a marijuana surrogate, have been traded through the Internet under the name of Spice/K2 since 2004. With the ban of 1st and 2nd generation SC, new 3rd generation SC have been introduced as Spice/K2 components. We selected four of these SC, namely BB-22, 5F-PB-22, 5F-AKB-48 and STS-135 and we studied their *in vitro* affinity and agonist properties for rat and mice CB1 receptors and *in vivo* stimulant properties on DA transmission in the rat nucleus accumbens (NAc) shell and core and medial prefrontal cortex (mPFC).

The four compounds bind with high affinity to CB1 receptors in rat cerebral cortex homogenates and stimulate CB1-induced [35S]GTPγS binding with high potency and efficacy. BB-22 and 5F-PB-22 showed the lowest Ki (0.11 and 0.13 nM), i.e., 30 and respectively 26 times lower than that of JWH-018 (3.38 nM), and a potency (EC50, 2.7 and 3.9 nM, respectively) and efficacy (Emax, 217% and 203%, respectively) as CB1 agonists higher than JWH-018 (EC50, 20 nM; Emax, 163%). 5F-AKB-48 and STS-135 had higher Ki for CB1 binding, higher EC50 and lower Emax as CB1 agonists than BB-22 and 5F-PB-22 but still comparatively more favourable than JWH-018. The agonist properties of all the compounds were abolished or drastically reduced in the presence of the CB1 antagonist/inverse agonist AM251 at concentrations (0.1 µM) that do not reduce basal activity. No activation of G-protein was observed in CB1-KO mice. BB-22 (0.003–0.010 mg/kg iv) increased dialysate DA in the NAc shell but not core or mPFC, with bell shaped dose-response curve and an effect at 0.01 mg/kg and a biphasic time-course. Administration of AM251 (1.0 mg/kg ip) prevented the stimulant effect of BB-22 on dialysate DA in the NAc shell. All the other compounds increased dialysate DA in the NAc shell at doses consistent with their *in vitro* affinity for CB1 receptors (5F-PB-22, 0.01 mg/kg; 5F-AKB-48, 0.1 mg/kg; STS-135, 0.15 mg/kg iv).

In conclusion, 3rd generation cannabinoids can be even more potent and effective CB1 receptor agonists than the original Spice/K2 component JWH-018. These compounds activate DA transmission in the NAc shell with potencies comparable to those *in vitro* as CB1 agonists. Future research will establish if these properties can explain the high acute toxicity and abuse liability of these compounds.

S16 Room: Marie Louise 1
NEW PERSPECTIVES IN ANIMAL MODELS OF DEPRESSION: PHARMACOLOGICAL, ANATOMICAL, ENDOCRINE AND BEHAVIORAL APPROACHES

Chairs: Mercè Correa, ES and John D. Salamone, USA

S16.1
COGNITIVE FLEXIBILITY: CORTICOSTRIATAL CIRCUITS AND NEUROCHEMICAL MODULATION IN RATS

Wolfgang Hauber
University of Stuttgart, Department Animal Physiology D-70569 Stuttgart, Germany

Animals including humans usually select appropriate actions to obtain specific goals or outcomes because they have knowledge about the causal relationships between predictive stimuli, actions and outcomes such as food. This allows flexible adaption of goal-directed action in an ever-changing environment and, therefore, efficient decision making and survival.

Many forms of decision making depend on the prefrontal cortex and the striatum and their interactions through multiple serial and parallel loops modulated by monoamines. Rodent studies suggest that specific subregions of the prefrontal cortex (PFC) and nucleus accumbens (NAc) are key components of an interconnected neural system that subserves effort- and risk-based decision making. For instance, in rats not only inactivation of the anterior cingulate cortex (ACC) and NAc but also disconnection between the ACC and the basolateral amygdala or between the ACC and the NAc impairs effort-based decision making. Thus, an information transfer between these structures is essential to guide decisions requiring an assessment of the costs such as effort expenditure relative to the benefits of obtaining reward. Furthermore, there is compelling evidence that mesolimbic dopamine (DA) regulates how much effort to invest for benefits such as food reward. For instance, relative to sham controls, rats with NAc DA depletion had a reduced preference for effortful but large-reward action. Moreover, rats subjected to DA receptor blockade in the core region of the NAc had a reduced preference for risky but large-reward action. By contrast, stimulant drugs such amphetamine increase the preference for effortful or risky large-reward action.

Of note, acute stress can also influence flexible decision making. For instance, an acute pharmacological stressor that involves combined administration of corticosterone and yohimbine, a selective α2-adrenoceptor antagonist, enhanced risky choice. In addition, acute

psychological stressors can alter flexible responding: a restraint stressor rendered performance of previously acquired instrumental action inflexible and habitual, possibly due to a compromised retrieval of encoded relationships between actions and their outcomes.

Impairments of flexible decision making have been observed in numerous psychiatric disorders including depression. Thus, an analysis of e.g. effort-related decision making in rodents may not only be important for an understanding of the neural and neurochemical basis of the underlying cognitive and motivational processes. It could also help to develop an animal model for dysfunctions related to effort expenditure in neuropsychiatric disorders such as depression.

S16.2
EFFORT-RELATED DECISION MAKING IN HUMAN DEPRESSION AND ANIMAL MODELS: DISTINCT ROLES FOR DIFFERENT MONOAMINES

John D. Salamone
Department of Psychological Sciences, University of Connecticut, Storrs, CT, USA 06269-1020

Motivational symptoms such as anergia, fatigue, or apathy are frequently observed in patients with depression, schizophrenia and other disorders. Depressed people show reduced selection of high-effort alternatives, and tasks that measure effort-based choice are being developed as animal models of motivational symptoms. In rodents, effort-based choice tasks allow animals to select between a more valued reinforcer that can only be obtained by a high degree of effort versus a low effort/low reward option. Low doses of dopamine (DA) antagonists and accumbens DA depletions shift choice behavior, decreasing selection of the high effort option and increasing choice of the low effort option. Alterations in effort-based choice in rodents are induced by conditions associated with depression, such as injection of tetrabenazine (TBZ), which blocks monoamine storage, and by pro-inflammatory cytokines (IL-1 and IL-6). The drug-induced deficits in effort-based choice can be used to assess the effects of known and potential therapeutic agents. Several drugs have been shown to reverse the effort-related effects of TBZ or cytokines, including the DA uptake blockers bupropion, GBR12909, methylphenidate, modafinil, PRX-14040, and lisdexamfetamine (LDX). The norepinephrine (NE) uptake blocker desipramine does not reverse the effects of TBZ, nor do the serotonin uptake blockers (SSRIs) fluoxetine or S-citalopram. The lack of effect of SSRIs is consistent with clinical reports showing that SSRIs are relatively ineffective for treating fatigue and anergia, and can exacerbate these symptoms. We also assessed the ability of drugs to increase work output in rats tested on a progressive ratio (PROG)/chow feeding choice task. Acute and repeated injections of bupropion, GBR12909, LDX and PRX-14040 increased PROG work output. In contrast, fluoxetine, desipramine, and the NE uptake blocker atomoxetine failed to increase lever pressing output, and actually decreased it at higher doses. Bupropion and GBR12909 at behaviorally active doses elevated extracellular DA in accumbens as measured by microdialysis, while fluoxetine, desipramine and atomoxetine did not. These results demonstrate that effort-related motivational symptoms can be modeled in rodents, and suggest a role for DA in regulating these symptoms.

S16.3
IMPACT OF EXERCISE ON THE DOPAMINERGIC SYSTEM AND ON SELECTION OF ACTIVE VS. PASSIVE SOURCES OF REINFORCEMENT: IMPLICATIONS FOR THE TREATMENT OF ANERGIA IN DEPRESSION

Mercè Correa
Psychobiology Dept. Universitat Jaume I. Castelló. Spain

S16.4
CHRONIC UNPREDICTABLE STRESS AS AN ANIMAL MODEL OF DEPRESSION: NEW DATA AND NEW PERSPECTIVES

Antonio Armario
Institut de Neurociències, Universitat Autònoma de Barcelona, Cerdanyola del Vallès 08193, Barcelona, Spain

The chronic unpredictable stress (CUS) model was developed by Katz et al. (1981) by exposing rats to different types of emotional and physical stressors over a period of 3 weeks. They consider this model close to chronic stress situations in humans in that they have an important degree of unpredictability. Later on, we and other demonstrated several behavioral and physiological consequences of CUS compatible with a putative animal model for depression, and Willner and coworkers, focusing on changes in the dopaminergic system and the development of anhedonia (evaluated by sucrose intake) greatly contributed to the validation of the model. However, despite their popularity and extensive use, there are some important concerns with this model: (i) the details about the type of stressors used vary considerably among the different laboratories; (ii) the results are very often inconsistent, with some reports of reduced anxiety after CUS. These inconsistencies could in great part due to the use of animals having different degrees of susceptibility, raising the question whether a

prior history of chronic stress could be detrimental or to the contrary protect from the consequences of novel stressors, depending on the characteristics of animals. Moreover, there is also evidence in the literature that a history of chronic stress can protect from some consequences of novel superimposed stressors. We then hypothesized that prior CUS exposure could protect at least for some of the negative consequences of later exposure to a severe stressor (immobilization on boards, IMO). Then, we have compared in a series of experiment how a prior history of chronic IMO and CUS (in which IMO was omitted) can alter the consequences of a single IMO session. Whereas prior chronic IMO exposure reduced the acute IMO-induced release of hypothalamic-pituitary-adrenal (HPA) hormones (ACTH, corticosterone), reflecting adaptation to the homotypic stressor, prior CUS modestly increased initial response to IMO but accelerated the post-IMO decline of plasma ACTH, suggesting a possible dual effect of CUS: sensitization of the initial response and enhanced post-IMO recovery. Importantly, prior chronic IMO reduced the negative impact of a single IMO session on activity in an open-field, saccharin intake and body weight, and also reduced active coping during IMO (struggling). Prior CUS partially protects from these negative effects of IMO, but struggling was similar to controls. Active coping behavior in the forced swim test was not reduced by chronic IMO but it was by CUS; however, CUS did not reduce saccharin intake, questioning the use of sucrose intake as an index of anhedonia. To gain insight into the brain areas mostly altered by prior CUS exposure we evaluated the effect of CUS on ΔFosB expression (an index of chronic neuronal activation) under resting conditions and on the c-fos response (an index of neuronal activation) to IMO in a wide range of brain areas. While chronic IMO induced ΔFosB accumulation in a reduced set of brain areas, CUS increased ΔFosB levels in the same areas, typically to a greater extent, but also in additional areas, including the medial prefrontal cortex. The greater and wider ΔFosB induction caused by CUS is suggestive a more complex consequences than IMO, although the functional implication of these findings remain to be clarified. Interestingly, prior CUS significantly reduced c-fos response to an acute IMO in an important number of brain areas as compared with stress-naïve rats, supporting the cross-adaptation observed with certain behaviors. Overall, the present results suggest that under certain conditions, prior CUS exposure might induce cross-adaptation to other novel stressors. The factors responsible for the direction of CUS-induced changes (resilience versus vulnerability) remain to be identified.

S17 Room: Marie Louise 2 MOTOR NEURON DISEASE: INSIGHTS INTO SPINAL MUSCULAR ATROPHY FROM MODEL ORGANISMS

Chair: Ruben J. Cauchi, MT

S17.1 THE ROLE OF SURVIVAL MOTOR NEURON PROTEINS (FL-SMN AND A-SMN) ON AXONAL FUNCTION AND MOTOR NEURON PHENOTYPE IN SPINAL MUSCULAR ATROPHY

Cinzia Cagnoli

Molecular Neuroanatomy and Pathogenesis Unit, Neurology VII, IRCCS Foundation Neurological Institute "C. Besta", via Temolo 4, 20126 Milano, Italy

Spinal Muscular Atrophy or SMA is a severe autosomal recessive disease characterized by selective motor neuron degeneration. SMA is the leading genetic cause of infant mortality, with an incidence of 1 in 6,000–10,000 neonates, a prevalence of 1 in 53,000 individuals, and an estimated carrier frequency of 1 in 35. In affected children, motor neuron loss leads to progressive amyotrophic paralysis, respiratory failure, and, in more severe cases, to death. SMA is classified into three main clinical types (I–III), in relation to the age of onset and disease severity. SMA is a monogenic disease caused by disruptions of the Survival of Motor Neuron 1 (*SMN1*) gene. The severity of the disease is related to the presence, peculiar to the human species, of the almost identical *SMN2* copy gene. The *SMN2* gene mainly expresses an exon 7 truncated SMN form (delta7-SMN) and only low amounts of the functional "full-length" FL-SMN protein. Thus, the presence of more *SMN2* copies may ameliorate the clinical course of SMA. In addition, a truncated form of SMN is expressed in neurons. This protein, termed axonal SMN (a-SMN), is translated from an *SMN1*-derived mRNA, which retains intron 3, containing a premature stop codon, and thereby produces a truncated shorter protein. Unlike FL-SMN, a-SMN has remarkable axonogenic properties, being capable of inducing time-dependent axon growth in both NSC34 cells and primary neurons. To study a-SMN function, we generated NSC34 clones with tetracycline-dependent a-SMN expression. All the a-SMN expressing clones were characterized by increased axonal thickness, elongation and branching if compared with the NSC34 controls. The analysis of a-SMN expression on cell motility demonstrated that the a-SMN clones were characterized by increased motility relative to the TR4 controls. In addition, whole-genome gene expression studies per-

mitted the identification of IGF1 (insulin-like growth factor-1), CCL2, and CCL7 (C-C motif ligands 2 and 7) as factors associated with a-SMN expression. Functional studies performed on CCL2 indicate that the chemokine contributes to the axonogenic and pro-motility action of a-SMN.

Finally, to verify the differential contribution of the two SMN protein isoforms to axon growth/neuronal differentiation, we used an *in vitro* setting, i.e., sandwich co-cultures of primary hippocampal neurons and glia. Embryonic hippocampal neurons in culture display well-defined and precise morphological steps of polarization and differentiation. By applying specific siRNAs efficiently down-regulating either FL-SMN or a-SMN proteins, we analysed the differential roles of FL-SMN and a-SMN in axon outgrowth and in neuronal homeostasis during differentiation of hippocampal neurons into a mature phenotype. The experiments provided two sets of data. First, by analyzing subcellular localization in primary cultures of hippocampal neurons, we showede that a-SMN is preferentially distributed in the axonal compartment since the very early phases of neuronal differentiation. Second, by specifically silencing FL-SMN or a-SMN proteins, we showed that both proteins play a role in axon growth, as their selective down-regulation reduces axon length without affecting dendritic arborization, and that a-SMN, but not FL-SMN, silencing is capable of inducing the growth of multi-neuritic neurons, i.e., neurons impaired in the differentiation process of selecting a single axon out of multiple neuritic processes.

Our results therefore indicate that a-SMN is able to influence the neuronal mechanisms of cell differentiation in the initial steps of axon growth, and support the role of FL-SMN in axon maintenance.

S17.2
EXPLORING THE FUNCTION OF THE SMN-GEMINS COMPLEX IN THE NEUROMUSCULAR SYSTEM THROUGH FRUIT FLY MODELS OF SPINAL MUSCULAR ATROPHY

Ruben J. Cauchi
Department of Physiology and Biochemistry, Faculty of Medicine and Surgery, University of Malta, Msida, Malta and Centre for Molecular Medicine and Biobanking, Biomedical Sciences Building, University of Malta, Msida, Malta

The neuromuscular disorder, spinal muscular atrophy (SMA), results from insufficient levels of the survival motor neuron (SMN) protein. Together with Gemins 2–8 and Unrip, SMN forms the large macromolecular SMN-Gemins complex whose best-characterised function concerns the assembly of spliceosomal small nuclear ribonucleoproteins (snRNPs). We made use of the fruit fly model system to explore whether disruption of this function is responsible for the selective neuromuscular degeneration in SMA. The fruit fly, *Drosophila melanogaster*, is a highly exploited genetic model organism for determining molecular mechanisms of human neurological disorders in view of the high degree of conservation at a genetic and biological level. Through loss-of-function analyses, we show that Gemin2, Gemin3 and Gemin5 are required for viability and optimal motor performance in *Drosophila*. Notably, the Gemin loss-of-function phenotypes mimic those induced on reduction of SMN levels, hence, indicating that absence of any member of the SMN-Gemins complex is sufficient to arrest the function of the complex. Importantly, we uncover that disruption of either pICln or Tgs1, two cardinal players in snRNP biogenesis, results in viability and motor phenotypes that closely resemble those uncovered on loss of the constituent members of the SMN-Gemins complex. We also demonstrate that viability and motor phenotypes associated with a hypomorphic Gemin3 mutant are enhanced by changes in the levels of SMN, Gemin2, Gemin5, pICln or Tgs1 brought about by various genetic manipulations within the muscle tissue. These findings confirm that members of the SMN-Gemins complex work closely with snRNP assembly factors *in vivo*. Interestingly, overexpression of either Gemin2, pICln or Tgs1 was by itself sufficient to cause motor dysfunction in *Drosophila*. Toxicity is conserved in the yeast *S. pombe* where overexpression induces a surplus of Sm proteins in the cytoplasm, indicating that a block in snRNP biogenesis is partly responsible for this phenotype. We propose that snRNP biogenesis is the pathway connecting the SMN-Gemins complex to a functional neuromuscular system, and its disturbance most likely leads to the motor dysfunction that is typical in SMA. Considering the intersection of the SMN-Gemins complex with amyotrophic lateral sclerosis (ALS) our findings have broader implications on our understanding of the mechanisms underpinning motor neuron disease.

S17.3
FUNCTIONAL ANALYSIS OF THE SMN COMPLEX AND IDENTIFICATION OF MODIFIER GENES IN FISSION YEAST

Remy Bordonné
IGMM-CNRS UMR5535, 1919 route de Mende, 34000 Montpellier, France

The biogenesis of the spliceosomal snRNPs is dependent on the Survival Motor Neuron (SMN) protein, the product of the Spinal Muscular Atrophy (SMA) disease gene SMN1. SMN homologues have been identified in higher eukaryotes as well as in the fission yeast *Schizosaccharomyces pombe*. We used the *S. pombe*

model organism to analyze the in vivo role of SMN in snRNP biogenesis and splicing. Fission yeast is of particular interest to study these processes because it contains a splicing machinery close to mammals in both similarity and content. To study more precisely the function of SMN in splicing, we constructed a temperature-degron fusion of SMN (tdSMN) in which the N-terminus of SMN is fused to the DHFR degron at its chromosomal locus. The tdSMN strain is viable but grows slower at 25 °C suggesting a deleterious effect of the tag already at this permissive temperature. We could demonstrate that SMN is directly required for the *in vivo* stability of the spliceosomal snRNAs associated with the Sm core complex. We also found that some introns are more sensitive to low snRNP levels than others. Similar defects have also been identified in fission yeast cells carrying a deletion of ICln, a component of the methylosome. Altogether, our data are consistent with the view that splice site selection and spliceosome kinetics are highly dependent on the concentration of core spliceosomal components.

Fission yeast has also been proven to be a useful model organism to identify genetic interactions, which can provide insights in finding functional modifier genes in more complex organisms. The characterization of such modifier genes appears particularly important in SMA because they could be able to modulate the phenotype of individuals. In order to characterize modifier genes connected to SMN, we performed an Epistatic MiniArray Profile (E-MAP) screen with the hypomorphic tdSMN mutant. As expected, our screen allowed the identification of critical regulators of snRNP assembly as for example the ICln protein involved in early steps of snRNP biogenesis. In addition, we identified genes linked to transcription by RNA polymerase II and to chromatin remodeling, consistent with close functional interplays between splicing, transcription and chromatin architecture. Our genetic screen allowed also the finding of several suppressors able to compensate the growth defect of the tdSMN mutant. These suppressors might represent valuable tools to decipher the molecular bases of rescue mechanisms in metazoan SMN-deficient cells.

S17.4
IDENTIFICATION OF NEUROPROTECTIVE MOLECULES FOR SPINAL MUSCULAR ATROPHY USING C. ELEGANS ANIMAL MODEL

Elia Di Schiavi
Institute of Bioscience and BioResources, CNR, Naples, Italy

Spinal muscular atrophy (SMA) is a genetic neuromuscular disorder caused by mutations in the evolutionary conserved Survival of Motor Neurons gene (*SMN1*). The loss of *SMN1* induces a selective degeneration of lower motor neurons, leading to progressive muscle atrophy and death. The molecular mechanisms underlying the disease are not understood, contributing to the lack of an efficacious treatment. In the absence of a specific molecular target, chemical screens can be performed using small model systems, that can, at the same time, elucidate the molecular basis of the disease and identify potential therapeutic compounds. *C. elegans* represents a valuable model organism for human diseases, since its genome encodes many human disease orthologs and the biological processes are well conserved. The small size, the rapid growth and its simple food requirements make the nematode a suitable model for pharmacological screenings on whole living animals.

To elucidate the molecular basis of SMA and identify lead compounds we therefore took advantage of a new SMA model developed in our laboratory, which is based on the transgene-driven interference of *smn-1*, the *C. elegans* homolog of *SMN1.* This genetic model is based on the expression, under a cell-specific promoter, of sense and antisense RNA molecules corresponding to fragments of *smn-1.* This RNAi strategy enabled us to efficiently reduce the function of *smn-1* gene specifically in 19 motor neurons. Transgenic lines in which *smn-1* is knocked down present an age-dependent degeneration of motor neurons that results in altered backward movements and in neuronal cell degeneration and death. Importantly, these animals are viable and fertile allowing us to overcome the lethality problem related to other *C. elegans* SMA models carrying *smn-1* loss of function alleles.

We have been using this model as a tool to discover natural extracts, natural compounds, synthetic drugs and genes presenting a neuroprotective function and thus able to rescue the cell death phenotype. We also setup the conditions to carry out an high-content screening of an FDA approved chemical library with 1200 compounds. Interestingly we found a number of conditions that can partially rescue cell death but not the onset of the degenerative process, while few others fully protect neuronal integrity and survival. By candidate gene approach and by random chemical mutagenesis we also identified some of the genetic interactors that, when mutated, are able to completely prevent neuronal death.

Neuronal degeneration underlies a variety of serious pathologies, which are largely unknown and that cause extreme personal discomfort and social costs. Uncovering the molecules and the pathways that prevent cell death in our SMA model may help design strategies aimed at preventing neurodegeneration in other pathologies. Some of the neuroprotective molecules identified using the SMA model have therefore been tested on different neurodegenerative models (e.g. axon degeneration, *huntingtin* or *alpha-synuclein* overexpression) and

indeed we found that a subset of these candidate molecules has a general protective role, while others are specific to our SMA model. By genetic manipulations, drug treatments and detailed phenotypic analysis we successfully identified specific genetic and chemical modifiers of *smn-1* function but also generic neuroprotective molecules.

S18 Room: Reading Room MOLECULAR INSIGHT INTO PAIN PERCEPTION

Chairs: Mark Landry, FR and Jacques Noël, FR

S18.1 PIEZO2 AND PROPRIOCEPTION

Ana Gomis
Instituto de Neurociencias. Universidad Miguel Hernández-Consejo Superior de Investigaciones Científicas. 03550 San Juan de Alicante, Spain

Nervous sensory terminals respond to mechanical forces evoking tactile and proprioceptive sensations when the stimulus exclusively activates mechanoreceptors, and painful sensations when nociceptors are activated. Significant progress has been made in understanding the cellular and molecular mechanisms of touch receptors however knowledge of proprioceptor mechanosensation is less well understood. Proprioceptors innervate muscle spindles, Golgi tendons and joints and mediate conscious sensation of limb position, muscle tension and balance.

Characterization of mechanotransducer channels indicates that Piezo2 is the principal mechanoreceptor in touch sensation. To determine the role of Piezo2 in proprioception, we have characterized the electrical and mechanical properties of neurons in the mesencephalic trigeminal nucleus (MTN), the unique primary sensory neurons located in the central nervous system. These neurons receive signals originating from the masseter muscle and dental pressoreceptors and are involved exclusively in processing proprioceptive information from the face and oral cavity. Neurons were identified by injecting the retrograde dye DiI into the masseter muscle. We performed whole-cell patch-clamp recordings, in voltage and current clamp configurations, in mice brain slices and we applied mechanical stimulation using a glass pipette driven by a micromanipulator system. The MTN neurons in mice respond to a current injection with a phasic discharge pattern. MTN neurons have a big soma size and show narrow action potentials, which are abolished by tetrodotoxin, a sodium channel blocker. All these characteristics are present in non-nociceptive neurons. MTN neurons only displayed rapidly adapting (RA) currents in response to mechanical indentation.

Piezo2 was detected in almost every MTN neuron by using immunohistochemistry thus, we explored the role of Piezo2 channels in mammalian proprioception. To abolish the expression of Piezo2 we silenced its expression by infecting MTN neurons of C57Bl/6j mice with an adeno-associated viral vector (AAV) carrying a shRNA against Piezo2. We also used a conditional Piezo2 KO mouse in which Piezo2 was eliminated exclusively in proprioceptive neurons. The results showed that RA mechanically-activated currents in proprioceptive neurons were fully dependent on Piezo2. Furthermore, functional characterization of Piezo2^{cKO} mice showed that the lack of Piezo2 in proprioceptive neurons produced severe defects in limb position and impaired performance in different balance and coordination behavioral tests. All those findings provided direct evidence that the ion channel Piezo2 is essential for mammalian proprioceptive mechanotransduction.

S18.2 REGULATION OF TRESK K2P CHANNEL AND ROLE IN PAIN SENSATION

Xavier Gasull
Neurophysiology Lab, Medical School, University of Barcelona, Institute of Neurosciences, University of Barcelona, Institut d'Investigacions Biomediques August Pi i Sunyer (IDIBAPS), Barcelona, Spain

The two-pore domain K+ channel TRESK/KCNK18 is expressed in DRG and trigeminal sensory neurons where, together with other K2Ps (TREK-1, TREK-2 and TRAAK) carries most of the total K+ background current. TRESK is modulated by pH changes, activated by inhaled anesthetics, inhibited by arachidonic acid and it is insensible to temperature changes. Alkylamides like hydroxy-alpha-sanshool and IBA (isobutylalkenyl amide), which inhibit TRESK channels, produce tingling, cooling and pungent burning sensations in humans and nocifensive behaviors in rats. We showed that peripheral axotomy decreases TRESK channel expression in DRGs, and pharmacological blockade or channel expression knockdown decreases mechanical threshold to painful stimuli and produces a nocifensive behavior in rats, which likely contributes to enhance sensory neuron excitability after injury. In addition, other researchers have identified mutations in TRESK that have been linked to the generation of familial migraine with aura. All these effects have implicated this channel in nociception and chronic pain. We described that TRESK currents are also modulated by shear stress, changing membrane tension (hypotonic swelling or cell shrinkage) or by applying membrane crenators or cup-formers. In addition to their regulation by Ca^{2+}/calcineurin activation, other factors such

as interaction with membrane lipids, lipid derivatives or channel trafficking are under study as possible modulators of TRESK activity. Besides, behavioral characterization of TRESK knockout mice shows that TRESK plays a significant role in the detection of noxious mechanical stimuli under normal and inflammatory conditions as well as in the response to inflammatory mediators such as prostaglandins or hypertonic stimulus. All together, we will present and discuss evidence showing that TRESK channels expressed in sensory neurons have a role in the transduction of nociceptive stimuli and constitute an interesting target for the development of analgesic drugs.

S18.3
SWITCH FROM EXCITATORY TO INHIBITORY MODULATION BY GROUP I METABOTROPIC GLUTAMATE RECEPTORS OF NOCICEPTIVE TRANSMISSION IN A MODEL OF INFLAMMATORY PAIN

Houda Radwani
University of Bordeaux, IINS, CNRS UMR 5297, Laboratory: central Mechanism of sensitization to pain. Bordeaux, France and University of Abdelmalek Essaâdi, Faculty of Sciences, Laboratory: Physiology and Physiopathology, Tetouan, Morocco

Pathological pain is a major social problem affecting quality of life and causing an important economical cost. The spinal cord is the first central relay for the treatment of nociceptive information. Dorsal horn neurons (DHNs) of the spinal cord express group I metabotropic Glutamate receptors (mGluRs I) which are known to modulate L-type calcium channels involved in sensitization to pain. In this study we aim at analyzing the role of mGluRs I and L-type calcium channels in spinal nociceptive transmission in naive and inflamed in adult Sprague-dawley rats. The role of mGluRs I in modulating nociceptive transmission through L-type calcium channels was evaluated by using behavioral and *in vivo* extracellular recording. We show that activation of group I mGluRs induce, in naïve anesthetized rats, an increase in C-fiber-evoked field potentials requiring the activation of L-type calcium channels. Very surprisingly, in inflamed rats, activation of mGluRs I induced inhibition of C-fiber-evoked field potentials independent of L-type calcium channels. Moreover, in awakes animals, pain behavior was also bidirectionally affected by mGluRs I activation in the spinal cord. We then sought for the underlying mechanism. First we show that inflammation does not alter the expression of mRNA neither for mGluRs I (mGluR 1 and mGluR 5) nor for L-type calcium channels (Cav1.2 and Cav1.3). Second, we seek for specific site of action mGluRs I and we show that the inhibitory effect of mGluRs I activation in inflammatory condition is completely suppressed after blocking the inhibitory neurotransmission.

These results suggest that mGluRs I activation decrease nociceptive transmission through activation of inhibitory network in the dorsal horn of the spinal cord in a model of persistent pain making mGluRs I a potential target for therapeutical approaches in a context of pathology.

S18.4
NON-ACIDIC ACTIVATION OF PAIN-RELATED ACID-SENSING ION CHANNEL 3 BY LIPIDS

Jacques Noël
Institut de Pharmacologie Moléculaire et Cellulaire, UMR 7275 CNRS - Université de Nice Sophia Antipolis, Université Côte d'Azur, France

Acid-Sensing Ion Channels (ASICs) are proton sensitive depolarizing sodium channels expressed throughout the nervous system. They are composed of the trimetric association of ASIC1 to ASIC3 subunits. Protons are the principal signal that activates ASICs channels, which are considered as sensors of extracellular pH variations. They are involved in various physiological processes associated with tissue acidification and play a major role in pain sensation. In the peripheral nervous system, we have shown the role of ASIC3 in inflammatory pain and muscular post-operative pain.

We observed that human inflammatory exudates, from patients with painful joint effusion, do not correlate with acidic pH, but rather have neutral pH 7.4. We show that these inflammatory exudates activate human ASIC3 channels generating a slow constitutive current. This is largely driven by lipids, and we identify lysophosphatidylcholine (LPC) and arachidonic acid (AA) as endogenous activators of ASIC3 in the absence of any extracellular acidification. The combination of LPC and AA evokes robust depolarizing current in DRG neurons through activation of ASIC3 channel, increases nociceptive C-fiber firing, and induces pain behavior in rats. All these effects are prevented by ASIC3 blockers and LPC and AA induced pain is also significantly reduced in ASIC3 knockout mice.

These findings open new perspectives on the roles of ASIC3 in the absence of tissue pH variation, as well as on the contribution of those channels to lipid-mediated signaling.

S19 Room: Ballroom
NEW INSIGHTS ON FUNCTIONAL INTEGRATION IN THE CORTICO-THALAMO-CORTICAL CIRCUIT

Chair: Vincenzo Crunelli, UK/MT

S19.1
THALAMOCORTICAL INTERACTIONS DURING SENSORY PROCESSING IN THE MAMMALIAN BRAIN

Alexander Groh
Technical University of Munich, Germany

The cerebral cortex is arguably the 'cognitive' headquarter of the brain thought to accommodate distinct cortical circuits for decision making, conscious perception and other cognitive functions. But how does the cortex communicate with the rest of the brain to fulfill these functions? Unlike the cortex, subcortical structures like the thalamus are located deep in the brain and are thus inaccessible with cellular imaging techniques, rendering the study of cortico-subcortical interactions a major challenge in neuroscience. I will talk about the long range interactions between the cortex and the thalamus studied with deep brain electrophysiology techniques in combination with optogenetics in the rodent somatosensory system. Thalamocortical interaction are bidirectional such that both structures provide major input to each other. As a consequence of bidirectional interactions between the cortex and the thalamus, sensory signals are differentially gated through the thalamocortical system depending on the previous and ongoing activity in the cortex and thalamus. This loop architecture allows the thalamocortical system to adapt sensory information processing in order to respond in a meaningful way to current requirements. Recent work on these networks emphasize the diverse functional effects of cortical feedback to the thalamus. For example, the activity in a substantial portion of the thalamus – the higher-order thalamus – is dominated by cortical inputs through sparse yet strong synapses which can be modulated by sensory input. In contrast, in a different corticothalamic feedback system, we found weak interactions between the cortex and the thalamus which support several forms of adaptive processing of touch signals. These examples demonstrate that sensory processing is dynamically controlled by long range synaptic interactions between the cortex and subcortical structures and provide a mechanistic basis to address more complex behavior related interactions between the cortex and the rest of the brain.

S19.2
MODULATION OF CA_V3-CHANNEL-DEPENDENT THALAMIC RHYTHMOGENESIS THROUGH CORTICORETICULAR SYNAPTIC PLASTICITY

Lüthi Anita
*University of Lausanne, Department of Fundamental Neurosciences, Rue du Bugnon 9, 1005 Lausanne, Switzerland *Present address: EPFL, Laboratory of Behavioral Genetics, 1015 Lausanne Switzerland*

Thalamic circuits are reliable and stereotypic pacemakers for rhythms and they substantially contribute to the patterning of cortical sleep waves. Yet, sleep oscillations vary in intensity, locally and globally in the brain, but the contribution of thalamic plasticity is unknown. We explored synaptically mediated modulation of nRt discharge using selective optogenetic activation of layer VI cortical afferents in mouse brain slices. We will describe a novel form of plasticity at these synapses that increases the propensity of reticular neurons to discharge in bursts upon cortical input and that could affect corticothalamic rhythmicity in the anesthetized mouse brain.

S19.3
RAPID BRAIN STATE DEPENDENT MODULATION OF SPONTANEOUS AND SENSORY EVOKED ACTIVITY IN THE LATERAL GENICULATE NUCLEUS OF AWAKE MICE

Magor L. Lőrincz
University of Szeged, Department of Physiology, Anatomy and Neuroscience, Közép fasor 52., Szeged, 6726, Hungary

In the absence of sensory input, the mammalian brain exhibits a wide array of structured, state dependent spontaneous activities. In cortical networks periods of active wakefulness are associated with depolarized membrane potential, asynchronous firing and fast oscillations in the local field potential, whereas periods of quiet wakefulness are asociated with hyperpolarized membrane potential, synchronous firing and large amplitude low frequency oscillations. The activity of thalamocortical neurons during these rapid brain state transitions has not yet been characterized. To this end, we performed multi-unit and local field potential recordings in the visual cortex with simultaneous extracellular or intracellular recordings of identified dorsal lateral geniculate nucleus (dLGN) neurons of awake, head restrained mice while monitoring their pupil size. The firing rate of some dLGN neurons showed clear correlations with the pupil size on a rapid time scale indicating that TC neurons exhibit brain state dependent activity changes. To reveal the effect of rapid state changes on sensory coding the response to visual stimuli (moving gratings of different orientations) was compared between periods of different pupil diameters. We found that the orientation tuning of some TC neurons is brain state dependent. These results indicate that the activity of TC neurons can change during brain state transitions on a rapid timescale resulting in altered sensory responses.

S20 Room: Carlson Suite
NEW INSIGHTS INTO BASAL GANGLIA CIRCUITRY: IMPLICATIONS FOR UNDERSTANDING AND TREATING BASAL GANGLIA DISORDERS

Chairs: Suzanne N. Haber, USA Hagai Bergman, IL

S20.1
ULTRA-HIGH FIELD MRI POST MORTEM STRUCTURAL CONNECTIVITY OF THE HUMAN SUBTHALAMIC NUCLEUS, SUBSTANTIA NIGRA, AND GLOBUS PALLIDUS

Yasin Temel
Departments of Neuroscience and Neurosurgery, Maastricht University Medical Center, Maastricht, The Netherlands

The subthalamic nucleus, substantia nigra, and globus pallidus, three nuclei of the human basal ganglia, play an important role in motor, associative, and limbic processing. The network of the basal ganglia is generally characterized by a direct, indirect, and hyperdirect pathway. Here, we will share our findings on the mesoscopic nature of these connections between the subthalamic nucleus, substantia nigra, and globus pallidus and their surrounding structures. We have scanned a human post mortem brain specimen including the substantia nigra, subthalamic nucleus, and globus pallidus was scanned on a 7 T MRI scanner. High resolution diffusion weighted images were used to reconstruct the fibers intersecting the substantia nigra, subthalamic nucleus, and globus pallidus. The course and density of these tracks was analyzed. We were able to reconstruct most of the commonly established projections of the subthalamic nucleus, substantia nigra, and globus pallidus. However, some of the reconstructed fiber tracks such as the connections of the substantia nigra pars compacta to the other included nuclei and the connections with the anterior commissure have not been shown previously. In addition, the quantitative tractography approach showed a typical degree of connectivity previously not documented. An example is the relatively larger projections of the subthalamic nucleus to the substantia nigra pars reticulata when compared to the projections to the globus pallidus internus. Our study shows that ultra-high field post mortem tractography allows for detailed 3D reconstruction of the projection of deep brain structures in humans. Although the results should be interpreted carefully, the newly identified connections contribute to our understanding of the basal ganglia.

S20.2
DIRECT AND INDIRECT CORTICAL INFLUENCE ON DOPAMINE NEURONS

Suzanne N. Haber
University of Rochester, Rochester, NY, USA

The basal ganglia (BG), play an important role in emotion, cognition, and motor control. The network is generally characterized by a massive cortical input primarily to the striatum. However, the cortex also influences other BG structures, either directly or indirectly. Of particular interest are the midbrain dopamine neurons and its relationship to cortical influences. These cells are reciprocally connected to the striatum, and as such receive a massive, but indirect cortical input via and striatum. Two other structures, the subthalamic nucleus and zona incerta (ZI) also receive direct cortical input, with both impacting on the dopamine cells. The ZI is of particular interest as it projects both directly to the dopamine neurons and to the lateral habenula. The lateral habenula, in turn, projects to dopamine cells via the mesopontine rostromedial tegmental nucleus (RMTg). This network is important in processing negative reward related signals. This talks will address the complexity of the various routes by which cortex can influence midbrain dopamine cells.

S20.3
THE SUBTHALAMIC NUCLEUS AND THE STRIATUM – DRIVING FORCE VS. FINE TUNING

Hagai Bergman
Department of Neurobiology (Physiology) and Neurosurgery, Edmond and Lily Safra center (ELSC) for Brain research, The Hebrew University – Hadassah medical center, Jerusalem, Israel

The basal ganglia (BG) main axis is built as three layers neural network, where the striatum and the subthalamic nucleus (STN) constitute the BG inputs and together innervate the BG downstream structures using GABA and glutamate, respectively. However, the respective contribution of these two distinct inputs in shaping BG downstream activity in health and parkinsonism is still unknown.

Comparison of the neuronal activity in BG input and downstream structures reveals that subthalamic, not striatal, activity fluctuations correlate with modulations in the increase/decrease discharge balance of BG downstream neurons during classical condition task with short and long delays. After striatal dopamine depletion and induction of parkinsonism with MPTP, ab-

normal low beta (8–15 Hz) spiking and local field potential (LFP) oscillations emerge and resonate across the BG network. Nevertheless, LFP beta oscillations entrain spiking activity of STN, striatal cholinergic interneurons and BG downstream structures, but do not entrain spiking activity of striatal projection neurons.

Together, these results highlight the pivotal role of STN glutamatergic and divergent projections in BG physiology and pathophysiology and provide sound explanation for the widespread choice of the STN as the prime target along the BG network for DBS in patients suffering from Parkinson's disease.

S20.4
THE NEUROPHYSIOLOGICAL SUBSTRATE OF NON-MOTOR DISORDERS IN THE CONTEXT OF PARKINSON'S DISEASE

Abdelhamid Benazzouz
Université de Bordeaux, Institut des Maladies Neurodégénératives, CNRS UMR 5293, 33076 Bordeaux, France

Despite the focus on motor deficits, Parkinson's disease is also characterized by non-motor symptoms, including anxiety and mood disorders, which are understudied and therefore not well treated. The motor symptoms are classically supposed to be originated from the degeneration of dopamine (DA) neurons in the pars compacta of substantia nigra (SNc) and the non-motor symptoms have been suggested to be originated from the dysfunction of the three monoaminegic systems. To verify this hypothesis, we investigated i) the impact of combined lesions of DA, norepinephrine (NE) and serotonin (5-HT) neurons on the manifestation of parkinsonian-like motor deficits, anxiety and mood disorders; ii) whether the combined lesions may interfere with the beneficial effect of L-Dopa; and iii) the role played by the basolateral amygdala (BLA) and the lateral habenula (LHb) in the observed behavioral effects. Concerning anxiety, we showed that selective bilateral lesion of SNc DA neurons induced anxiety-like disorder and that additional lesion of locus coeruleus NE and/or dorsal raphe 5-HT neurons did not potentiate this anxiogenic effect. L-Dopa significantly improved anxiety behavior in animals with bilateral lesion of DA neurons alone or combined with NE cell lesion. This improvement was paralleled by an increase in the firing rate of BLA neurons in the two animal groups. However, in animals with combined lesions of DA and 5-HT neurons or in animals with lesions of the three monoaminergic systems, L-Dopa did not improve anxiety behavior, and in parallel did not change the electrophysiological parameters of BLA neurons. These results provide the first evidence that lesioning 5-HT neurons, but not NE neurons, alters the beneficial effect of L-Dopa on anxiety and the changes in the electrical activity of BLA neurons. Concernig mood disorders, as for anxiety, we showed that selective bilateral DA cell lesion alone induced depressive-like behavior and anhedonia and that additional depletions of NE and/or 5-HT did not potentiate this effect. We also showed that additional selective lesions of 5-HT neurons in the dorsal raphe induced a lack of motivation similar to apathy reported in PD patients. L-Dopa reversed depressive-like behavior in rats with bilateral DA cell lesion alone or combined with the NE and/or 5-HT depletion. However, L-Dopa had no effect on anhedonia and apathy. We also showed a link between depressive like behavior and the neuronal activity of LHb as L-Dopa treatment increased the response of LHb neurons in parallel with the efficacy of L-Dopa on depressive-like behavior. Our data highlight the important role of DA cell degeneration in the manifestation of depressive-like disorder and provide the first evidence that apathy, insensitive to the replacement of DA by L-Dopa, is not dependent solely to DA depletion but to the combination of DA and 5-HT cell degeneration.

S21 Room: Marie Louise 1
Cannabinoid regulation of the mesocorticolimbic dopamine system: Implications for neuropsychiatric disorders

Chair: Steven Laviolette, CA

S21.1
UNDERSTANDING THE DIFFERENTIAL EFFECTS OF THC AND CANNABIDIOL ON MESOLIMBIC DOPAMINE FUNCTION: IMPLICATIONS FOR THE ETIOLOGY AND TREATMENT OF SCHIZOPHRENIA

Steven R. Laviolette
Dept. of Anatomy & Cell Biology, Dept. of Psychiatry, Schulich School of Medicine & Dentistry, University of Western Ontario. London, Ontario, Canada

Marijuana represents a highly complex plant with diverse phytochemical constituents. The primary psychoactive compound in marijuana, delta-9-tetrahydrocannabinol (THC), has been shown in both clinical and pre-clinical studies to possess potent pro-psychotic properties, particularly following exposure during adolescent neurodevelopment. In contrast, the largest non-psychoactive compound in marijuana, cannbidiol (CBD), has been shown to functionally and pharmacologically counteract the effects of THC, and more importantly, possesses promising anti-psychotic properties. Our laboratory is characterizing the neuronal, behavioural and molecular effects of both THC and CBD directly within the

mammalian mesocorticolimbic system. Thus far, we have discovered that while acute or neurodevelopmental exposure to THC can dramatically dysregulate dopamine (DA) transmission in the mesocorticolimbic circuit, CBD can strongly counteract these effects, and dampen hyperactivity of DAergic transmission. While THC induces schizophrenia-like abnormalities in affective and cognitive processes and schizophrenia-like molecular adaptations in mesocorticolimbic structures like the prefrontal cortex and nucleus accumbens; CBD produces opposite effects, and can potently reverse schizophrenia-like abnormalities directly in the mesocorticolimbic system. While the effects of THC are dependent upon interactions with DA receptor transmission, CBD acts through a distinct molecular pathway mediated through the serotonin 5-HT_{1A} receptor system. Specifically, our evidence demonstrates that the differential regulation of the glycogen synthase kinase-3 (GSK-3), mammalian target of rapamycin (mTOR) and p70S6-kinase signaling pathways by THC vs. CBD is most likely responsible for the opposing neuropsychiatric effects of THC and CBD, respectively. This presentation will review our most recent evidence showing that THC and CBD produce their pro vs. anti-psychotic properties through distinct and in some cases, opposing molecular pathways and neuronal control mechanisms directly in the mesocorticolimbic circuitry.

S21.2
UTO PLEASE OR NOT TO PLEASE? THE ENDOCANNABINOID SYSTEM AND ITS INVOLVEMENT IN REWARD AND ADDICTION WITH A FOCUS ON INTRACRANIAL SELF-STIMULATION STUDIES

Styliani Vlachou
School of Nursing and Human Sciences, Faculty of Science and Health, Dublin City University, Ireland

The endocannabinoid system is thought to modulate the motivational processes and reward-seeking behaviours associated with the (ab)use of cannabis. However, cannabinoids appear atypical as drugs of abuse and have shown controversial and/or biphasic effects in *in vivo* behavioural studies focusing on their reinforcing properties.

The most commonly used behavioural paradigms for the investigation of the reinforcing properties of drugs of abuse, including cannabinoids, are the intracranial self-stimulation (ICSS), the conditioned place preference (CPP) and the intravenous self-administration (IVSA) procedures.

This talk will present a summary of findings from studies using the ICSS, IVSA and CPP procedures, with a strong focus on results from the ICSS studies, many of which coming from early work by Vlachou and colleagues.

The possible reasons for and implications of the controversial and/or biphasic effects of cannabinoid compounds and discrepancies seen among these studies will be discussed.

S21.3
INFLUENCE OF JWH-018 REPEATED ADMINISTRATION ON THE RESPONSIVENESS OF MESOLIMBIC AND MESOCORTICAL DOPAMINE TRANSMISSION TO REWARDING STIMULI

Maria Antonietta De Luca
Department of Biomedical Sciences, University of Cagliari, Italy

In the past decade numerous "herbal mixtures" containing synthetic cannabinoids, broadly known as *Spice*, have been marketed as legal alternatives to *Cannabis*. The synthetic cannabinoid 1-pentyl-3-(1-naphthoyl)-indole (JWH-018) has been detected in about 140 samples of Spice. JWH-018 is a CB1 and CB2 agonist with a higher affinity than Δ9-THC, the active ingredient of Marijuana. Recently, we showed that JWH-018 has CB1-dependent reinforcing properties and increases dopamine (DA) transmission in the shell of the nucleus accumbens (NAc), similarly to Δ9-THC and to the synthetic cannabinoid WIN 55,212-2.

Previous studies of our group showed also that taste stimuli increase extracellular DA in the NAc and in the medial prefrontal cortex (mPFC) of rats. This effect shows single-trial habituation in NAc shell but not in core or in mPFC. However, morphine sensitization and mPFC 6-OHDA lesions, abolishes habituation of DA responsiveness to taste stimuli in NAc shell but induces it in mPFC. These observations support the hypothesis of an inhibitory influence of mPFC DA on NAc DA, and its putative role the loss of control of the motivational value of stimuli and in impulsivity. In addition, other studies showed that the chronic use of cannabinoids in humans is able to modulate the activity of mPFC producing a reduction of its functions, which are associated with amotivational syndrome, neurochemical hypofrontality, and dysfunctions of cortic-limbic-striatal DAergic circuit. The aim of this study was to investigate if the repeated administration of JWH-018 is associated to changes in the behavioral taste reactions to appetitive stimuli and in the response of DA transmission in the NAc shell and core and mPFC to the same stimuli. To this end, rats were administered once a day for 14 consecutive days with JWH-018 (0.25 mg/kg i.p.) or with vehicle. After a week of washout, rats were implanted with an intraoral catheter for the infusion of appetitive

stimuli, and microdialysis probes for *in vivo* analysis of DA dialysates. The day after, rats were infused intraorally with chocolate (1 ml/5 min). The behavioral taste reactions were recorded while the DA extracellular levels measured in the NAc shell and core and mPFC.

Our results showed that JWH-018 administration inhibits the NAc shell DA increase to a novel rewarding stimulus (i.e chocolate), abolished habituation of DA responsiveness to repeated chocolate exposure and induced it in the mPFC; while in the NAc core, JWH-018 treatment potentiated, delayed and prolonged the stimulatory DA response to taste stimuli.

No differences in behavioral taste reactivity have been observed. These results show that JWH-018 is able to induce differential adaptive changes of the responsiveness of DA transmission to taste stimuli in DA terminal areas, similarly to previous results obtained in morphine sensitized and mPFC 6-OHDA lesioned rats. Further studies on the neuronal damage produced by a repeated JWH-018 administration are in progress.

This study will be helpful to understand the possible detrimental effects (e.g. acute psychosis) of chronic use of Spice drugs, which represents a major public health concern.

S21.4
THE EFFECTS OF SYNTHETIC CANNABINOIDS ON EXECUTIVE FUNCTION AND RELATED BRAIN ACTIVITY IN fMRI

Aviv Weinstein
Department of Behavioral Science, University of Ariel, Ariel, Israel

There is an increasing use of novel psychoactive substances (NPs) worldwide containing cathinones and synthetic cannabis which is raising major public health concerns. Clinical studies have shown that chronic use of NPs affects the nervous system and can induce paranoid psychosis and hypomanic illness with grandiose delusions similar to amphetamine. Synthetic Cannabinoid (SC) products reported have effects similar to those of cannabis.

The purpose of the study was to assess whether chronic use of synthetic cannabinoids is causing impairment to cognitive function in chronic users. In the first phase of the study we have used computerized executive function tests- the Stroop, N-back test and a memory task in a group of twenty synthetic cannabinoids users, twenty recreational cannabis users and twenty healthy control subjects. Results showed that synthetic cannabinoid users performed worse than both control groups on the N-back task (more errors), the Stroop task (overall slow responses) and the long-term memory task (fewer word recall). They have also showed higher ratings of depression and trait-state anxiety compared with both control groups. In the second phase of this study we run fMRI studies in a group of synthetic cannabinoid users and control participants while performing executive function tasks. Results indicated impairment in behavioral performance on the N-back task and the G-No-Go task in SC users. Control participants employed the frontal, parietal, thalamus and the cerebellum whereas SC users did not show such activation.

to the best of our knowledge this is the first study showing cognitive and brain function impairment in synthetic cannabinoid users compared with regular users of cannabis and healthy control participants. This may have major implications for our understanding of the long-term consequences of novel psychoactive substances.

S22 Room: Marie Louise 2
NEUROLOGICAL MECHANISMS OF THE IMPACT OF STRESS ON MEMORY AND EMOTIONALITY IN HEALTH AND DISEASE: INSIGHTS FROM ANIMAL AND CLINICAL STUDIES

Chairs: Patrizia Campolongo, IT and Maria Morena, CA

S22.1
STRESS AND EMOTIONAL AROUSAL EFFECTS ON MEMORY

Benno Roozendaal
Dept. Cognitive Neuroscience, Radboud University Nijmegen Medical Centre; Donders Institute for Brain, Cognition and Behaviour, Radboud University Nijmegen, The Netherlands

Stressful or emotionally arousing experiences are typically well remembered. In my presentation, I will describe findings from animal experiments indicating that stress hormones (epinephrine and glucocorticoids) released from the adrenal glands are crucially involved in enhancing the consolidation of memory of emotionally arousing life experiences. In contrast, these stress hormones impair the retrieval of memory processing. Stress hormones do not uniformly modulate memory of all kinds of information but, rather, preferentially influence the consolidation and retrieval of memory of emotionally arousing information or during emotionally arousing test situations. These findings fit well with extensive evidence from our laboratory indicating that emotional arousal-induced noradrenergic activation within the basolateral complex of the amygdala (BLA) is critically involved in mediating such stress hormone effects on memory consolidation and memory retrieval.

Evidence that lesions of the BLA or infusions of a beta-adrenoceptor antagonist into the BLA block the modulatory effects of stress hormone administration on memory suggests that arousal-induced noradrenergic activation within the BLA is a co-requirement in enabling stress hormone effects to modulate memory consolidation. In turn, BLA activation regulates neural plasticity and information storage processes via its efferent projections to many other brain regions. Such arousal-induced BLA activation not only influences the strength of memory but also has important effects on accuracy of memory.

S22.2
MODULATION OF COGNITION AND EMOTION BY THE ENDOCANNABINOID SIGNALING IS DEPENDENT ON STRESS AND AROUSAL STATE

Maria Morena
Hotchkiss Brain Institute, Dept. of Cell Biology and Anatomy, University of Calgary, Calgary (AB), Canada; Dept. of Physiology and Pharmacology, Sapienza University of Rome, Rome, Italy

The activation of neuromodulatory systems by stress and emotional arousal within limbic brain regions plays a key role in the modulation of learning and memory for emotionally significant experiences. Cannabinoid type 1 (CB1) receptors are abundantly expressed within these brain regions, where they regulate emotional responses. Emerging evidence indicates that cannabinoid drugs can induce distinct and often opposite effects on anxiety, cognition, and several other behaviors, depending on the stress level and the aversiveness of the environmental context. Indeed, deliberate variations in environmental conditions have been demonstrated to result in marked changes in the effects of the same manipulations within the same series of experiments.

Findings will be presented on how states of high and low emotional arousal and different levels of stress at the time of training or testing influence endocannabinoid release in different limbic brain regions leading to differential effects on memory and emotionality. Data will show that CB1 antagonism induces opposite effects on recognition memory consolidation depending on the level of emotional arousal at the time of training and that the endocannabinoid 2-arachidonoylglycerol, in the hippocampus, is involved in the selective regulation of memory retrieval of stressful experiences. Furthermore, data will be presented on how increasing endocannabinoid anandamide and 2-arachidonoylglycerol levels in the amygdala selectively decreases anxiety when animals are tested under conditions of low emotional arousal.

Collectively, these findings help to elucidate the neural underpinnings of the fine-tuned regulation of limbic neurocircuitry involved in modulating the impact of stress and emotional arousal on memory processes and emotionality, thus facilitating our understanding of the state-dependency of many drug interventions on psychiatric disorders.

S22.3
GLUCOCORTICOIDS AND ENDOCANNABINOIDS: BIOMARKERS AND THERAPEUTICS FOR TRAUMATIC MEMORIES AND STRESS-RELATED DISORDERS – OR NOTHING AT ALL?

Gustav Schelling
Department of Anaesthesiology, University of Munich (LMU), Germany

Preclinical evidence has clearly shown that the consolidation and retrieval of traumatic memories is regulated by an interaction between the noradrenergic, the glucocorticoid and the endocannabinoid system. The endocannabinoid system is an important regulator of HPA-axis activity during stress, an effect which has also been demonstrated in healthy humans and in patients with PTSD. Likewise, a single nucleotide polymorphism (SNP) of the glucocorticoid receptor (GR) gene (the BclI–SNP), which enhances the sensitivity of the glucocorticoid receptors to cortisol and possibly HPA-axis feedback function, was associated with enhanced emotional memory performance in healthy volunteer. The presence of the BclI–SNP increased the risk for traumatic memories and PTSD symptoms in patients after ICU therapy and was linked to lower basal cortisol levels. A number of small studies have demonstrated that the administration of cortisol to critically ill or injured patients results in a significant reduction of PTSD symptoms after recovery without influencing the number of traumatic memories. The variability of cortisol levels between individuals and PTSD patients in particular is too high, however, to allow the use of saliva or plasma measurements of cortisol as a biomarker for stress-related disorders. Endocannabinoid plasma concentrations also vary greatly in patients with PTSD and both increased and decreased values were reported when PTSD patients were compared to healthy individuals. Furthermore, the endocannabinoid system is highly stress sensitive and plasma concentrations could be influenced by the emotional context of PTSD diagnoses and blood sampling. An alternative approach for endocannabinoid measurements in individuals with stress-related disorders is hair analysis which offers the possibility to analyze endocannabinoids incorporated into different hair segments over time. Recent studies have shown that this approach is technically feasible and demonstrated a negative relationship between hair en-

docannabinoid concentration and PTSD symptom intensity. There was, however, a considerable overlap in concentrations between traumatized individuals and patients after trauma who developed PTSD.

Although a valid biomarker for the diagnosis of PTSD would be highly desirable, none of the presently available compounds are of sufficient validity. Hair appears as an attractive matrix for biomarker development but validation studies need to be done in large and well-defined cohorts of individuals with stress-related disorders.

S22.4
THE ROLE OF GLUCOCORTICOIDS IN THE STRIATUM ON EMOTIONAL MEMORY

Ginna L Quirarte

Departamento de Neurobiología Conductual y Cognitiva, Instituto de Neurobiología, Universidad Nacional Autónoma de México, Campus Juriquilla Querétaro 76230, México

Extensive evidence indicates that glucocorticoid hormones enhance memory consolidation of emotional arousing tasks, and that the basolateral nucleus of the amygdala (BLA) is involved in regulating such glucocorticoid influences on memory consolidation. Glucocorticoid-induced memory enhancement can be blocked by intra-BLA infusion of ß-adrenergic antagonists. Data from our laboratory demonstrate that corticosterone administration into the dorsal striatum produces an enhancement of memory consolidation that can be blocked by concurrent administration of the β-blocker atenolol into the BLA. Additionally, other studies from our group have described that the activation of glucocorticoid receptors in the medial or lateral region of the striatum hinders or enhances consolidation, respectively, depending on the type of memory (spatial or procedural). It is also known that glucocorticoids impair the retrieval of spatial memory and this impairing effect depends on the noradrenergic system of the BLA. In addition, recent findings from our laboratory revealed that glucocorticoids in the dorsal striatum impair the retrieval of a procedural-like cued water maze task. These findings indicate that noradrenergic activation of the BLA is required for enabling striatal glucocorticoid actions in mediating the enhancing and impairing effects of glucocorticoids on consolidation and retrieval of procedural memory.

S22.5
STRESS, GENES AND EMOTIONAL MEMORY: IMPLICATIONS FOR FEAR-RELATED DISORDERS

Dominique J.-F. de Quervain

Division of Cognitive Neuroscience, University of Basel, Switzerland

Stress hormones have a profound impact on learning and memory. I will talk about the memory-modulating effects of glucocorticoids and the clinical implications with regard to the treatment of anxiety disorders and addiction. In particular, I will argue that the inhibiting effect of glucocorticoids on the retrieval of aversive memory and the enhancing effect on memory extinction are beneficial with regard to alleviating symptoms. Moreover, these effects on memory make glucocorticoids a good candidate for combining with psychotherapy. Finally, I will present genetic and epigenetic findings related to stress hormones, emotional memory and PTSD.

S23 Room: Clermont Suite
NEW ENDEAVOURS FOR AN ANCIENT STRUCTURE: NOVEL ROLES FOR THE CEREBELLUM IN PHYSIOLOGY AND PATHOLOGY

Chairs: Daniela Caruelli, IT and Marta Asunta Miquel Salgado-Araujo (ES)

S23.1
VIEWING THE PERSONALITY TRAITS THROUGH A CEREBELLAR LENS

Laura Petrosini

Department of Psychology, University "Sapienza" of Rome, Italy

Personality multidimensional traits comprise the cognitive, emotional, and behavioral characteristics and their variance in the expression appears to be linked to structural variance in specific brain regions. In evidencing associations between personality factors and neurobiological measures, neuroimaging literature engages the cerebellum in the context of contemporary theories of affective and cognitive cerebellar function. By using region of interest (ROI)- and voxel-based approaches, we evidenced that the cerebellar volumes correlate with the scores obtained in the Temperament and Character Inventory by Cloninger. Namely, cerebellar volumes are associated positively with Novelty Seeking scores and negatively with Harm Avoidance scores. Subjects who search for new situations as novelty seekers do (and harm avoiders do not do) show a different engagement of their cerebellar circuitries to rapidly adapt to changing environments. This emerging model of cerebellar functionality may explain how the cerebellar abilities in planning, controlling, and putting into action the behavior are associated to personality constructs. Interestingly, increased cerebellar volumes are even associated with high scores in alexithymia, construct of personality characterized by impairment in cognitive, emotional,

and affective processing. Thus, the cerebellar substrate for some personality dimensions extends the relationship between personality and brain areas to a structure up to now thought to be involved mainly in motor and cognitive functions, much less in emotional processes and even less in personality individual differences. On such a basis, it seems necessary to go over the traditional cortico-centric view of personality and to address the cerebellar function in sustaining aspects of motivational network that characterizes the different temperamental traits.

S23.2
CEREBELLA COMES TO THE BALL: COGNITIVE EVOLUTION AND THE CEREBELLUM

Robert A. Barton
Evolutionary Anthropology Research Group, Durham University, UK

Much attention has focused on the dramatic expansion of the forebrain, particularly the neocortex, as the neural substrate of higher cognition and its evolution. The cerebellum, however, contains about four times more neurons than the neocortex, and its size covaries with cognitive abilities both across and within species. Once differences in the scaling of connectivity in neocortex and cerebellum are accounted for, a marked and general pattern of correlated evolution of the two structures is apparent, reflecting their structural and functional integration. One deviation from this general pattern of correlated evolution is a relative expansion of the cerebellum in great apes (including humans) and other species with skilled and socially learned foraging techniques. The confluence of these comparative patterns, studies of ape foraging skills and social learning, and recent evidence on the cognitive neuroscience of the cerebellum, suggest an important role for the cerebellum in the evolution of the capacity for planning, execution and understanding of complex behavioural sequences – including tool use and language.

S23.3
PURKINJE CELLS LACKING PTEN: THE AUTISTIC CEREBELLUM

Daniela Carulli
Department of Neuroscience, Neuroscience Institute of Turin (NIT), University of Turin, Regione Gonzole 10, 10043 Orbassano (Turin), Italy and Neuroscience Institute of the Cavalieri-Ottolenghi Foundation (NICO), University of Turin, Regione Gonzole 10, 10043 Orbassano (Turin), Italy

Autism spectrum disorders (ASDs) are neurodevelopmental disorders characterized by impaired social interaction, isolated areas of interest and insistence on sameness. Mutations in *Phosphatase and tensin homolog* (*PTEN*) have been reported in individuals with ASDs. Recent evidence highlights a crucial role of the cerebellum in the etiopathogenesis of ASDs. In the present study we analyzed the specific contribution of cerebellar Purkinje cell (PC) *PTEN* loss to these disorders. Using the Cre-loxP recombination system, we generated conditional knockout mice in which *PTEN* inactivation was induced specifically in PCs. We investigated PC morphology and physiology as well as sociability, repetitive behavior, motor learning and cognitive inflexibility of adult PC *PTEN* mutant mice. Loss of *PTEN* in PCs results in autistic-like traits, including repetitive behavior, impaired sociability and deficits in motor learning. Mutant PCs appear hypertrophic and show structural abnormalities in dendrites and axons, decreased excitability, disrupted parallel fiber and climbing fiber synapses and late-onset cell death. Our results unveil new roles of *PTEN* in PC function and provide the first evidence of a link between the loss of *PTEN* in PCs and the genesis of ASD-like traits.

S23.4
THE CEREBELLUM'S ROLE IN ADDICTION. HAVE WE BEEN IGNORING THE ELEPHANT IN THE ROOM?

Marta Miquel
Área de Psicobiología. Universitat Jaume I. Castellón, Spain

An increasing amount of data suggests the involvement of the cerebellum in many of the brain functions affected in drug addicts. However, this structure has been ignored by the traditional point of view about addiction. The most recent research from our lab has tackled the investigation of the cerebellum's role in drug-dependent conditioned memories and self-administration. We have found two distinctive cerebellar hallmarks of preference for cues linked to cocaine experience. Cocaine-induced preference conditioning increased neural activity, as well as the perineuronal nets (PNN) surrounding Golgi inhibitory interneurons, at the apex of the granule cell layer. These signatures were not found when animals do not exhibit preference conditioning. Moreover, the history of drug self-administration seems to be a crucial factor to the regulation of Golgi PNNs. Long access to cocaine persistently induced the formation of fully condensed PNNs around Golgi neurons. This effect was not found after short access to the drug. Remarkably, Golgi neurons are key elements for the control of synaptic plasticity at the level of the granule cell layer.

In a different set of experiments we performed a series of lesion studies to assess the effect produced by the impairment of medial prefronto-cerebellar networks in

the acquisition of cocaine-induced preference conditioning. Either the inactivation of the infralimbic cortex or a lesion in the apex of the posterior vermis increased up to 100% the percentage of animals acquiring conditioned preference for cocaine, aside from modulating plasticity events in the other structure. A simultaneous inactivation of both sites prevented this effect of disinhibition. Motivation for food self-administration appears to be also affected by the cerebellar lesion. Therefore, our findings indicate that the dorsal region of the posterior vermis may be an important node of networks for the inhibitory control of goal-directed behaviour.

S23.5
WHY WE DANCE BETTER THAN ROBOTS

Chris I. De Zeeuw
Department of Neuroscience, Erasmus Medical Center, 3015 GE Rotterdam, and Institute for Neuroscience, Amsterdam, The Netherlands

Although our ability to store semantic declarative information can nowadays be readily surpassed by that of simple personal computers, our ability to learn and express procedural memories still outperforms that of supercomputers controlling the most advanced robots. To a large extent, our procedural memories are formed in the cerebellum, which embodies more than two-thirds of all neurons in our brain. In this review, we will focus on the emerging view that different modules of the cerebellum use different encoding schemes to form and express their respective memories. More specifically, zebrin-positive zones in the cerebellum, such as those controlling adaptation of the vestibulo-ocular reflex, appear to predominantly form their memories by potentiation mechanisms and express their memories via rate coding, whereas zebrin-negative zones, such as those controlling eyeblink conditioning, appear to predominantly form their memories by suppression mechanisms and express their memories in part by temporal coding using rebound bursting. Together, the different types of modules offer a rich repertoire to acquire and control.

S24 Room: Reading Room
THE EVOLUTION OF NEURONAL NETWORKS: INSECTS IN NEUROBIOLOGICAL STUDIES

Chairs: Martin Giurfa, FR and Hans-Joachim Pflueger, DE

S24.1
ON THE CONTROL OF LOCOMOTION IN INSECTS AND THE IMPORTANCE OF NEUROMODULATION

Hans-Joachim Pflüger
Freie Universität Berlin; Institute of Biology, Neurobiology; Königin-Luise-Str. 28-30, D-14195 Berlin, Germany

In the nervous system neuromodulators are the essential elements of plasticity including those of learning and memory, and often the behavioural context of their release is evolutionary conserved. Here, the actions of the biogenic amines octopamine and tyramine, important neuromodulators of the insect nervous system, are studied with respect to their functional role in arousal, stress responses and inducing locomotion. The two biogenic amines act coordinately on both the central and the peripheral nervous system. There is evidence that the two biogenic amines may cause opposite reactions and, thus, the relative concentrations of both amines may exert very fine control over their targets. The advantage of the insect nervous system is that once the distribution of aminergic neurones in the nervous system is known by the use of respective antibodies, the activity of single neurones or groups of neurones can be recorded during behaviour. It will be shown how octopamine and tyramine act in the various compartments of an insect and how they affect synaptic transmission, metabolism, and also whole neuronal networks in order to adapt behaviour to functional requirements. The insect species that are used in these studies range from large insects such as locusts, moths, stick insect and cockroaches to small insects such as fruit flies.

S24.2
THE TASTE OF PUNISHMENT: GUSTATORY LEARNING AND DISCRIMINATION IN HONEY BEES

Martin Giurfa
Research Center on Animal Cognition, Center for Integrative Biology, University of Toulouse; CNRS, UPS, France

Insects have provided fundamental insights into our understanding of sensory perception. Among insects, the honey bee Apis mellifera has a model status for studies on vision and olfaction due to its capacity to learn and discriminate stimuli of these modalities if they are associated with sucrose reinforcement. Yet, the gustatory sense of bees has remained largely unexplored due to the unavailability of conditioning paradigms in which tastes act as stimuli to be learned and discriminated rather than as reinforcements. Here we characterized taste perception in this insect by establishing the first gustatory conditioning protocol in which tastes are dissociated from their traditional role of feeding reinforcement and act as predictors of punishment. We took advantage of the sting extension reflex, which is elicited in harnessed bees by a mild electric shock. We

paired this aversive stimulus with tastants delivered to the antennae and trained bees to discriminate punished from non-punished tastes. Bees successfully learned and memorized taste-shock associations and even acquired defensive SER to innately preferred food. They discriminated efficiently between broad taste categories but not within taste categories, and were unable to differentiate bitter substances from distilled water. We explored the neural bases of gustatory SER conditioning in the bee brain and focused on aminergic pathways traditionally assigned to reinforcement representation. We found that associating sucrose and shock requires convergence of octopaminergic and dopaminergic signaling, thus showing that these pathways do not always mediate pure reinforcement signaling. Our results build on the overwhelming learning capacities of bees and reveal previously unknown aspects of honey bee taste. They bring new perspectives for the analysis of the bees' feeding decisions in natural and artificial landscapes, and reaffirm the value of an insect model to uncover basic principles of gustatory processing.

S24.3
ELUCIDATING OLFACTORY CODING MECHANISMS WITHIN AND BEYOND THE *DROSOPHILA* ANTENNAL LOBE

Silke Sachse
Max Planck Institute for Chemical Ecology, Department of Evolutionary Neuroethology, Jena, Germany

Animals use sensory systems to navigate the environment in a way that optimizes their survival and reproduction. The olfactory system plays here a major role in encoding chemical information and translating the outside world into a neuronal representation that enables an animal to take odor-guided decisions. We are investigating how odors are encoded and processed in the *Drosophila* brain to lead to a specific odor perception. The vinegar fly represents a premier model system for studying olfactory processing mechanisms since it exhibits a stereotyped architecture which is similar to its mammalian counterpart, but is less complex and highly tractable as well as susceptible to genetic manipulations. By exploiting these genetic techniques and linking them to neurophysiological, molecular and behavioral methods, we are dissecting the neural circuits that are involved in coding, processing and perception of odors. As a key method, we are using functional imaging to monitor activity patterns elicited by odors in the antennal lobe, the first olfactory neuropil, as well as in higher processing centers, such as the lateral horn. Moreover, we employ genetic tools to selective trace individual neurons for anatomical reconstructions. The talk will summarize our recent insights into coding strategies of the olfactory system regarding odor identity, hedonic valence as well as odor intensity.

S24.4
ON BEHAVIORAL AND NEURAL MANIPULATIONS OF INSECTS BY PARASITIC WASPS

Frederic Libersat
Dept. of Life Sciences and Zlotowski Center for Neurosciences Ben Gurion University, POB 653 Beer Sheva, Israel

Neuro-parasitology is an emerging branch of science that deals with parasites that can control the nervous system of the host. The ability of parasites to alter the behavior of their hosts has recently generated an unusual interest in both scientists and non-scientists. Although the alteration of host behavior by parasites is a widespread phenomenon, underlying mechanisms are only beginning to be deciphered. The most fascinating examples of behavioral manipulation are seen in arthropods parasitized by various species of parasitoid wasps. These wasps manufacture venoms to manipulate the host nervous system in ways that are tailored to the developmental needs of their offspring. The parasitoid jewel wasp hunts cockroaches to serve as a live food supply for its offspring. The wasp stings the cockroach in the head and delivers a neurotoxic venom cocktail directly inside the prey's cerebral ganglia to apparently 'hijack its free will'. Although not paralyzed, the stung cockroach becomes a living yet docile 'zombie' incapable of self-initiating walking or escape running. In this presentation, we will show that the venom contains components that are aimed at modulating specific circuits to the benefit of both the wasp and its offspring. In this respect, the wasp is taking advantage of neurotransmitters and neuromodulatory systems present in the host to induce a sequential and adaptive behavioral manipulation.

S25 Room: Marie Louise 1
BRIDGING THE GAP: NOVEL DEVELOPMENTS IN THE TREATMENT OF EPILEPSY

Chair: Janet Mifsud, MT

S25.1
THE ROLE OF EUROPEAN FUNDED RESEARCH IN BRIDGING THE GAP IN EPILEPSY

Janet Mifsud
Department of Clinical Pharmacology and Therapeutics, University of Malta Msida MSD2040 Malta

The translation of basic neuroscience and genetic findings to the epilepsy clinic remains a major challenge, especially with respect to identification of the correct target population to be tested, the availability of biomarkers for patient stratification and prediction of treatment response, and optimization of study designs. Recent EU funded projects have shown that creating a critical mass of researchers in epilepsy in Europe and the Mediterranean can lead to such breakthroughs.

A search was undertaken in various databases and EU sites for projects which include epilepsy in their research aims.

Several laboratories and research institutions in Europe are supported from the EU for research on the mechanisms of epileptogenesis and on the development of new therapies. Currently, these account for a total EU investment of about 70 million euros. Three main key words in these projects include treatment, diagnosis/biomarkers and genetics, or gene therapy.

Building a framework for epilepsy research in Europe and the identification of opportunities for the future is important to keep the momentum of epilepsy research. It is key to share latest findings in epilepsy research; disseminate the results; meet stakeholders to discuss research developments and priorities to define common initiatives in order to identify opportunities for the future and to ensure that the infrastructures generated in the framework of these projects remain sustainable.

S25.2
PHARMACOGENETICS OF LAMOTRIGINE METABOLISM IN PAEDIATRIC POPULATIONS

Christian Saliba
Centre for Molecular Medicine and Biobanking, University of Malta

Paediatric populations with epilepsy offer a specific challenge in dose individualisation since they undergo significant developmental changes from childhood to adulthood. Thus, pharmacogenetics is extremely useful in the individualisation of drug therapy. This study has investigated the pharmacogenetics of a novel antiepileptic drug, lamotrigine, in a paediatric population. The aim of this study was to investigate the influence of genetic polymorphisms in metabolising enzymes, responsible for the metabolism of lamotrigine, on the drug concentration in Maltese paediatric patients with epilepsy.

Blood samples from 17 Maltese paediatric patients were collected. Genomic DNA was extracted using well established standard protocols. Primers were designed to span the entire coding sequence of the *UGT1A4* and *UGT2B7* genes. DNA amplification of the specific genes was performed by PCR on the respective patient samples. Gel electrophoresis was used to confirm that the desired DNA fragment was obtained. The PCR products were then purified and sequenced using the Sanger sequencing method.

This study investigated the possible correlation of metabolising enzymes such as UGTs, to population pharmacokinetics of antiepileptic drugs, and use of such genotyping in the individualisation of drug therapy of novel antiepileptic drugs in the clinical paediatric scenarios. Various mutations were identified in both genes of interest, leading to amino acid changes in the majority of patients. Genetic polymorphisms of UGTs result in different phenotypes by affecting expression levels or activity of individual UGT enzymes, hence variability in drug metabolism and elimination. This may result in inter-individual variability in pharmacokinetics of lamotrigine.

In humans, lamotrigine is primarily metabolised by *UGT1A4* and *UGT2B7*. This metabolism seems to be dependent on ethnicity. Genetic polymorphisms of these UGTs have an impact on metabolism and thus lamotrigine pharmacokinetics and contribute to inter-individual variability.

This research will lead to the improvement of therapeutic outcomes for paediatric populations with epilepsy, by understanding the contribution of pharmacogenetics to the individualization of drug therapy and development of personalised medicine.

S25.3
PRECISION MEDICINE IN THE EPILEPSIES

Sanjay Sisodiya
UCL Institute of Neurology, UK

Treatment for many epilepsies remains suboptimal. As the genetic causes of many of these epilepsies, there is growing hope for effective, individualised 'precision medicine'. This concept is largely driven from a genetic perspective. Genetics is one component of the description of most epilepsies, and itself may be more complex than a single gene defect alone. Additional sources of information, including imaging, data on unexplained comorbidities, and functional data, will also contribute to shaping treatment at the individual level.

Data suggest that in a few instances, genetic findings can directly guide effective treatment. In other cases, treatment predicted to be effective does not work, a phenomenon requiring explanation. These observations will be discussed during the presentation.

Precision medicine in the epilepsies holds much promise, but it is also important that expectations are real-

istic and take genomic and phenotypic complexity into account.

S25.4
THE GENETIC EPILEPTIC ENCEPHALOPATHIES: FROM MOLECULAR DIAGNOSIS TO MANAGEMENT STRATEGIES

Renzo Guerrini
University of Florence, Italy

Next Generation Sequencing (NGS) has contributed identifying a large number of monogenic epilepsy syndromes and is favoring earlier and more accurate diagnosis in a subset of paediatric patients with epilepsy.

The cumulative information emerging from NGS studies is rapidly changing our comprehension of the relations between early onset severe epilepsy and the associated neurological impairment, progressively delineating specific entities previously gathered under the umbrella definition of epileptic encephalopathies, thereby influencing treatment choices and limiting the most aggressive drug regimens only to those conditions that are likely to actually benefit from them.

Although ion channel genes represent the gene family most frequently causally related to epilepsy, other genes have gradually been associated with complex developmental epilepsy conditions, revealing the pathogenic role of mutations affecting diverse molecular pathways that regulate membrane excitability, synaptic plasticity, presynaptic neurotransmitter release, postsynaptic receptors, transporters, cell metabolism, and many formative steps in early brain development. Some of these discoveries are being followed by proof of concept laboratory studies that might open new pathways towards personalized treatment choices. While for most of the monogenic disorders that can now be diagnosed early using NGS no specific treatment is available and etiological diagnosis, better prognostication and genetic counseling are the only benefits deriving from knowing the specific cause, for a limited number of them timely treatment based on their known molecular pathology is already possible, and sometimes decisive.

Discovery of a causative gene defect associated with a nonprogressive course may reduce the need for further diagnostic investigations in the search of a progressive disorder at the biochemical and imaging level. NGS has also improved the turnaround time for molecular diagnosis and allowed more timely and straightforward treatment choices for specific conditions as well as avoidance of needless investigations and withholding inappropriate or unnecessary treatment choices.

S26 CANCELLED Room: Carlson Suite NEURONAL VULNERABILITY AND SYNUCLEINOPATHIES – NEW INSIGHTS IN PARKINSON'S DISEASE

Chair: Jochen Roeper, DE

S26.1
HABITUAL AND GOAL DIRECTED BEHAVIOR IN A PARKINSON DISEASE MODEL

Ledia F. Hernandez
HM-CINAC – Hospital Universitario HM Puerta del Sur, San Pablo-CEU University, Madrid, Spain

Humans can combine automatic habitual and voluntary goal-directed tasks simultaneously with consummate ease. In everyday life, frequent switching between these two modes of behavioural control occurs, as task components encountered are predictable or uncertain. Experimental evidence from both animal and human investigations indicates that the dopaminergic neurons of the substantia nigra pars compacta (SNpc) that innervate the sensorimotor striatum are essential for the acquisition of automatized skills and habits, and are activated when automatic habitual control is deployed. In Parkinson Disease the predominant loss of dopamine innervation occurs in the sensorimotor putamen, which has been implicated in habitual control. We hypothesize that the reliance on habitual performance, which is prevalent in much of human behaviour, may be a key vulnerability factor for preferential degeneration of the nigro-striatal sensorimotor projection. We discuss this hypothesis in the light of recent anatomical, functional and electrophysiological evidence showing that, compared with their more medially located counterparts, dopamine neurons in the lateral substantia nigra have more diffusely branching terminal fields, are activated by a greater range of sensory events, and are engaged whenever behaviour is under more automatic or habitual control. Our proposal is that such factors impose essential additional stress on these metabolically vulnerable dopamine neurons, which over long lifetimes, promotes their degeneration, with the consequent impairment of automatic habitual control commonly experienced by patients with Parkinson disease. I will present my previous work on basal ganglia engagement during motor skill learning task and data from other groups supporting our hypothesis.

S26.2
MECHANISMS OF ACQUIRED VULNERABILITY OF SUBSTANTIA NIGRA DOPAMINE NEURONS IN ALPHA-SYNUCLEIN MODELS OF PARKINSON'S DISEASE

Jochen Roeper
Institute of Neurophysiology, Neuroscience Center,

Goethe University Frankfurt, Germany

Parkinson disease (PD) is an α-synucleinopathy resulting in the preferential loss of highly vulnerable dopamine (DA) substantia nigra (SN) neurons. Mutations (e.g., A53T) in the α-synuclein gene (SNCA) are sufficient to cause PD, but the mechanism of their selective action on vulnerable DA SN neurons is unknown. In a mouse model overexpressing mutant α-synuclein (A53T-SNCA), we identified a SN-selective increase of in vivo firing frequencies in DA midbrain neurons, which was not observed in DA neurons in the ventral tegmental area. The selective and age-dependent gain-of-function phenotype of A53T-SCNA overexpressing DA SN neurons was in part mediated by an increase of their intrinsic pacemaker frequency caused by a redox-dependent impairment of A-type Kv4.3 potassium channels. This selective enhancement of "stressful pacemaking" of DA SN neurons *in vivo* defines a functional response to mutant α-synuclein that might be useful as a novel biomarker for the "DA system at risk" before the onset of neurodegeneration in PD. We also present new evidence that in addition to redox-dependent dimerization of Kv4.3 proteins, the subunit composition of the Kv4.3 complex is selectively altered in the SN of A53T-SCNA animals. We currently aim to study the consequences of A53T-SNCA-induced Kv4.3 dysfunction in awake behaving animals.

S27 Room: Ballroom
DEVELOPMENT OF CANNABINOID-BASED THERAPIES TO PRESERVE NEURONS AGAINST ACUTE OR CHRONIC DAMAGE

Chair: Jochen Roeper, DE

S27.1
CANNABINOIDS AND EPIGENTIC REGULATION IN NEURODEGENERATIVE DISORDERS

Mauro Maccarrone
Department of Medicine, Campus Bio-Medico University of Rome, Via Alvaro del Portillo 21, 00128 Rome, Italy. European Center for Brain Research/Santa Lucia Foundation IRCCS, Via del Fosso di Fiorano 64, 00143 Rome, Italy

Endocannabinoids (eCBs) are endogenous lipids able to activate cannabinoid receptors, the primary molecular targets of the cannabis (Cannabis sativa) active principle Δ9-tetrahydrocannabinol, and other non-cannabinoid targets. During the last twenty years, several ethanolamides and esters of fatty acids (in particular arachidonic acid) have been shown to act as eCBs, and a complex array of receptors, metabolic enzymes, transmembrane and intracellular transporters (that altogether form the so-called "eCB system") has been shown to finely tune their manifold biological activities. Accumulated evidence has demonstrated that epigenetic regulation (i.e., modulation of gene expression by DNA methylation, histone modifications and/or microRNAs) is a major driver of eCB signaling in human neurodegenerative disorders like Alzheimer's disease, Parkinson's disease, multiple sclerosis, and amyotrophic lateral sclerosis [1, 2]. Here, an overview of the impact of eCB system in these disorders will be summarized, along with the state of the art of its epigenetic regulation. The aim is to put in a better perspective the potential of "epigenetic drugs" as more effective therapeutics to combat eCB-related human neurodegeneration.

S27.2
CANNABINOIDS FOR THE TREATMENT OF ALZHEIMER'S DISEASE

Ester Aso
Institut de Neuropatologia, Servei d'Anatomia Patològica, Hospital Universitari de Bellvitge, L'Hospitalet de Llobregat; Centro de Investigación Biomédica en Red de Enfermedades Neurodegenerativas (CIBERNED), Spain

Alzheimer's disease (AD), the most frequent cause of dementia, is an age-dependent neurodegenerative process morphologically characterized by the presence in brain of amyloid-β (Aβ) deposition and neurofibrillary tangles, mainly composed of hyper-phosphorylated tau protein. The progression from early stages of the neurodegenerative process to symptomatic stages may take decades, whereas once the cognitive impairment and dementia appear the disease progression is much more rapid. Therefore, AD is a relatively well-tolerated degenerative process during a long period of time, but it may have devastating effects once thresholds are crossed. These facts highlight the need to search for treatments that act on selective targets during the earliest stages of the disease, aimed at curbing or retarding disease progression toward dementia.

During the last few years, targeting the endogenous cannabinoid system has emerged as a potential therapeutic approach to treat AD. Cannabinoids may target in parallel several processes that play key roles in this neurodegenerative disease, including Aβ and tau aberrant processing, chronic inflammatory responses, excitotoxicity, mitochondrial dysfunction and oxidative stress, among others. Thus, the activation of both CB1 and CB2 cannabinoid receptors by natural or synthetic agonists, at non-psychoactive doses, have demonstrated to have beneficial effects in AD experimental models by reducing the harmful Aβ peptide action and tau phosphorylation, as well as by promoting the brain's intrinsic repair mechanisms. Interestingly, the administration of

a combination of two botanical extracts derived from the Cannabis sativa plant, which are the components of an already approved cannabis-based medicine, results in cognitive improvement together with reduction of several pathologic parameters in APP/PS1 transgenic mice, a well-established murine model of AD-related pathology, both at early symptomatic and at more advanced stages of the disease progression. These findings have prompted the progression towards a phase II clinical trial, which is currently under development, to evaluate the safety of this cannabis-based medicine in patients at early stages of AD and secondarily to test the efficacy of cannabinoids to curb some pathological markers associated to this neurodegenerative disease. The successful completion of this clinical trial might be the first step towards the use of cannabinoids against AD.

S27.3
THE ENDOCANNABINOID SIGNALING PROVIDES DRUGGABLE TARGETS FOR NEUROPROTECTION: AN OVERVIEW

Javier Fernández-Ruiz
Departamento de Bioquímica y Biología Molecular, Facultad de Medicina, Instituto Universitario de Investigación en Neuroquímica, Universidad Complutense, Madrid, Spain; Instituto Ramón y Cajal de Investigación Sanitaria (IRYCIS), Madrid, Spain

Cannabinoids form a singular family of plant-derived compounds, endogenous signalling lipids and synthetic derivatives with multiple biological effects and therapeutic applications in the CNS. One of these properties is the regulation of neuronal homeostasis and integrity, which is the result of a combination of effects addressed to preserve, rescue, repair and/or replace neurons and also some glial cell subpopulations (e.g. astrocytes, oligodendrocytes and their precursor cells) against those insults that damage these cells. This is critical in the CNS in which neuron loss is extremely difficult to overcome as neurons are post-mitotic cells incapable of replicating their DNA and dividing. Lost neurons may be replaced by new neurons, although orchestrating the generation of these neurons from neural progenitor cells in the adult brain is still limited. These limitations make the preservation of the original neurons generated during brain development or naturally replaced during the lifetime of the individual, a key objective to ensure the correct functioning of the different brain structures. The neuroprotective effects of cannabinoids are facilitated by the location of specific targets for the action of these compounds (e.g. CB1 and CB2 receptors, endocannabinoid inactivating enzymes, non-endocannabinoid targets) in key cellular substrates (e.g. neurons, astrocytes, resting and reactive microglia, perivascular microglial cells, oligodendrocytes and oligodendrocyte precursor cells, and neural progenitor cells) and structures (e.g. blood-brain barrier (BBB)). Such cellular locations enable cannabinoid compounds to exert selective control over the specific functions fulfilled by these cells in degeneration, protection, and/or repair. This should facilitate the development of a multitarget strategy which is essential in neurodegenerative disorders, in which neuronal damage is the result of a concerted action of different neurotoxic processes, e.g. excitotoxicity, oxidative stress, protein aggregation, mitochondrial failure, glial reactivity, inflammation. Individual cannabinoids, and also combinations of some of them, may function as broad-spectrum agents, being efficacious in the attenuation of such concerted neurotoxic actions. For example, CB1 receptors located in glutamatergic neurons may be activated to normalize glutamate homeostasis then limiting excitotoxic damage. CB1 receptors located in astrocytes may be useful to enhance trophic support of these cells for neurons, whereas such receptors may also facilitate the remyelination processes by promoting oligodendrocyte maturation from their precursor cells. Their location in BBB cells may improve vascular supply and limit peripheral cell infiltration to the CNS. CB2 receptors are also located in glial cells, in particular when they become activated, in which they control the toxic influence of these reactive cells (e.g. generation of fractalkine from astrocytes, or proinflammatory cytokines from microglial cells) for neurons. These receptors are also located in the BBB (e.g. perivascular microglial cells) then also contributing to improve the vascular supply and limit cell infiltration. Additional endocannabinoid targets, such as the degradative enzymes FAAH and MAGL, are present in neurons but also in astrocytes, whereas novel neuroprotective targets relatively close to the endocannabinoid system, such as the orphan receptor GPR55 or the nuclear receptors of the PPAR family, have been also identified in key cellular substrates (e.g. neurons, microglial cells) playing an anti-inflammatory and neuroprotective activity. Lastly, cannabinoid receptor-independent antioxidant/anti-inflammatory effects, in part mediated by acting on some transcription factors (e.g. Nrf-2, NF&B), have been also involved in the neuroprotective effects of certain cannabinoid compounds. All these capabilities support the broad-spectrum activity of cannabinoid compounds, then placing the endocannabinoid system in a promising position for developing novel neuroprotective therapies and demanding a prompt clinical validation of cannabinoid-based medicines for the treatment of different neurodegenerative disorders, which, at present, lack of efficacious treatments for delaying/arresting disease progression.

S27.4
PRECLINICAL AND CLINICAL STUDIES WITH CANNABINOIDS IN HUNTINGTON'S DISEASE

Onintza Sagredo
Departamento de Bioquímica y Biología Molecular, Facultad de Medicina, Instituto Universitario de Investigación en Neuroquímica, Universidad Complutense, Madrid, Spain; Centro de Investigación Biomédica en Red de Enfermedades Neurodegenerativas (CIBERNED); Instituto Ramón y Cajal de Investigación Sanitaria (IRYCIS), Madrid, Spain

Huntington's disease (HD) is a genetic disorder caused by an excessive number of CAG repeats in the gene encoding the regulatory protein huntingtin. The key symptoms in HD are choreic movements, which are produced by the degeneration of the striatum, and behavioral disturbances and dementia, which are caused by deterioration in cortical structures. Pharmacological therapies for HD patients are extremely limited, with only the inhibitor of the VMAT1 tetrabenazine (Xenazine®) approved for the treatment of choreic movements, but with poor results in most of patients. Certain cannabinoids formulated as Sativex® have been recently evaluated in HD patients. This was supported by an exhaustive preclinical work with positive results in a broad spectrum of animal models of HD (e.g. R6/2 mice, quinolinate-lesioned mice, 3-nitropropionate- or malonatelesioned rats) that confirmed the benefits of cannabinoids against most of the cytotoxic stimuli acting in this disease. For example, compounds targeting the CB1 receptor preserved striatal neurons in studies conducted in a rat model that relies on quinolinate-induced excitotoxic damage. The relevance of these receptors in HD was also demonstrated in a genetic model of the disease, R6/2 mice, in which CB1 receptor activation again preserved striatal neurons from death, whereas striatal damage was aggravated in R6/2 mice having a genetic deficiency in CB1 receptors. Compounds that selectively activate the CB2 receptor also appear to be effective in HD, preferentially ameliorating the inflammatory events and microglial activation that occurs after the striatum is damaged with malonate (a complex II inhibitor) in rats, in R6/2 mice and following the excitotoxicity induced by striatal lesion with quinolinate in mice. These effects may be facilitated by the overexpression of CB2 receptors in activated glial cells recruited at the striatal parenchyma, an effect that was first detected when striatal damage was provoked in rats with malonate and furtherly in R6/2 mice and other genetic mouse models of HD, as well as in post-mortem tissues from HD patients. Antioxidant non-psychoactive phytocannabinoids, such as cannabidiol (CBD) and cannabigerol (CBG), have also been investigated in experimental models of HD, even though its effects are independent of CB1/CB2 receptors. Their effects may be mediated by activation of PPARs or other non-endocannabinoid targets. CBD was very active in animal models characterized by mitochondrial damage, oxidative stress and calpain activation, such as rats intoxicated with the complex II inhibitor 3-nitropropionate. CBG was neuroprotective in 3-nitropropionate-lesioned and R6/2 mice. Based on these beneficial effects, CBD combined with Δ9-THC, as in the cannabinoidbased medicine Sativex has also been studied in animal models of HD given the wide-spectrum of pharmacological actions produced by this combination. This combination preserved striatal neurons in malonate lesioned mice and in 3-nitropropionate-lesioned rats. Cannabinoids have been also examined in HD patients, although the first clinical trials concentrated in the alleviation of specific symptoms, particularly chorea and behavioral disturbances, with controversial results. The only clinical trial aimed at validating a cannabinoid-based neuroprotective therapy in HD has been recently carried out in Spain using Sativex®, and, althought it successfully demonstrated that Sativex® was safe and well-tolerated in HD patients, as previously found in controls, yet unfortunately, it failed to provide any evidence that it may slow down disease progression in HD. This may be related to the relatively short time (12 weeks) for the active treatment and an unexpected influence of the placebo effect, so it is possible that a longer time may be necessary for revealing neuroprotective effects in HD patients treated with Sativex or similar preparations. This remains as one of the major challenge for the clinical validation of a cannabinoid-based medicine in HD.

S27.5
CANNABINOIDS FOR THE TREATMENT OF PARKINSON'S DISEASE

Antonio Pisani
Neurology, Dept. Systems Medicine, University of Rome "Tor Vergata", Rome, Italy

Current views of basal ganglia dysfunction point to the striatum as a primary locus of the pathophysiological changes underlying movement disorders, such as Parkinson's disease (PD). Of note, the striatal endocannabinoid system undergoes significant chemical and physiological changes after dopamine loss, and this has been reported both in animal models as wellas in PD patients. We performed an electrophysiological characterization of corticostriatal synaptic activity and plasticity by using whole-cell patch-clamp and conventional recordings of striatal spiny projection neurons (SPNs) from corticostriatal slices of mice lacking the PTEN-induced kinase 1 (PINK1) gene and in their wild-type littermates (PINK1+/+). While CB1

receptor activation reduced spontaneous glutamatergic EPSCs and GABAergic IPSCs in PINK1+/+ mice, CB1 receptor agonists were unable to modulate either glutamate-mediated or GABAergic spontaneous currents in PINK1−/− mice. Evoked corticostriatal EPSP amplitude was unaffected following CB1 receptor activation. Parallel biochemical measurements revealed no significant changes of 2-arachidonylglycerol (2-AG) and anandamide (AEA) content. A complete rescue of the CB1-dependent inhibition of synaptic activity was obtained with either amphetamine or the D2R agonist quinpirole. Our data demostrate show a selective CB1 receptor dysfunction in PINK1−/− mice, restored by dopamine replacement, supporting the evidence of a close, dynamic interaction between endocannabinoid and dopaminergic systems.

S28 Room: Clermont Suite FUNCTIONAL CONNECTIVITY STUDIES ON MOTOR AND LANGUAGE LEARNING WITH HEALTHY AND BRAIN DAMAGED ADULTS

Chair: Ana Inés Ansaldo, CA

S28.1 FUNCTIONAL CONNECTIVITY IN FMRI: AN OVERVIEW OF CONNECTIVITY METHODS

Benali Habib

ECE Dpt- PERFORM Centre, Concordia University, Canada, and Laboratoire d'Imagerie Biomédicale, INSERM U1146 - CNRS UMR 7371-UPMC UM CR2, Pierre & Marie Curie University, Paris, France

During the last decade, several brain systems have been studied in functional MRI using functional connectivity related approaches. Recently, compelling experimental evidence has been brought to support the fact that the brain contains several anatomically distinct large scale neural networks, including primary systems and associative networks. Exploratory approaches have also allowed to extract several functional networks at once. Even if all brain areas are not included in a network, these networks involve many areas and constitute a possible functional parcellation of the brain. It has been suggested that low frequency fluctuations i (< 0.1 Hz) of functional magnetic resonance imaging (fMRI) time-series acquired in healthy volunteers at rest characterize these networks. To robustly detect these large-scale neural networks, two categories of methods may be identified for such studies: approaches that make use of prior cognitive information and fully exploratory methods. Correlational methods were historically the first ones to be applied to investigate large-scale networks in fMRI data analysis, in the form of functional connectivity studies and functional connectivity maps. The fully exploratory methods provides data-driven approaches of large- scale network detection. They include methods based on eigenvalue decomposition, such as principal component analysis, neural network, hierarchical clustering, integration and information-theoretic quantities and spatial independent component analysis (sICA). Outstandingly, sICA has been used quite a lot recently, with results that are rather promising but many questions remain open regarding what methodologies to apply to extract functional networks. Methods developed so far for effective connectivity, such as structural equation modeling (SEM) or dynamical causal modeling (DCM) have been of little use to the investigation of extended large-scale networks. Methods originating from graph theories might prove more adapted to such problems. I will introduce the rationale and methodological concepts behind functional connectivity methods, including operational definitions, measures, pre-processing and processing data approaches. A set of exploratory techniques, including sICA, SEM and Graph Theory will be discussed.

S28.2 AGE-RELATED CHANGES IN FUNCTIONAL CONNECTIVITY FOR WORD PRODUCTION

Yves Joanette

Centre de recherche, Institut universitaire de gériatrie de Montréal Faculté de médecine, Université de Montréal, Canada

Neurodegenerative diseases associated with aging, such as Alzheimer's disease, affects progressively all cognitive domains resulting in the clinical condition referred to as dementia. However, despite the importance of the incidence of dementia in our societies, the majority of older people – even the oldest among them – do not exhibit dementia and have relatively preserved cognitive abilities, allowing them to maintain their contribution to society. Numerous studies have described the changes in cognitive abilities that characterize the later stages of life. These changes have been captured in concepts such as cognitive and motor slowness, or limited working memory. These characteristics, however, have been described using approaches and behavioural tools that have first been developed for younger adults. Consequently, the literature on cognitive abilities in older adults essentially focuses on what is less, or wrong, in older adults by reference to younger adults. But it becomes more and more obvious that despite the impact of the trajectory of life on the efficiency of some cognitive components, other cognitive components may in fact benefit from the life-long learning and accumulation of knowledge. This is the case for language abil-

ities, and mostly for semantic knowledge. Indeed, language abilities are among the few cognitive components that have been recurrently described as relatively preserved in aging. And there is evidence that there can even be life-long gains made, such as the enhanced richness of semantic knowledge with aging. Consequently, the semantic component of language abilities represents a unique window on the competing life-long opposing forces seeing the interaction between losses of some cognitive efficiencies on one side, and, on the other side, enrichment of knowledge, strategies and approaches which might correspond to the notion of wisdom associated with aging in some cultures.

This is why the goal of our research program is to understand the neurofunctional bases of the life-long source of tensions on cognitive abilities status in aging. Language abilities and particularly its semantic component, have been chosen to look at these changes for the reason delineated above, namely that this component of language represents a unique example of an ability whose efficiency is maintained, and even enhanced, with aging. And one of the best known semantic ability is certainly the semantic processing of isolated words. This is why our group has looked at the neurofunctional reorganisation of the semantic processing of words in aging. Brain imaging was of course to be used, but the classical approaches in which the neuroimaging techniques simply points to the contribution of discrete areas of the brain was felt insufficient to capture the richness of the changes in aging. Consequently, it was decided to complement classical approaches of functional neuroimaging of the semantic processing of words in aging by adding analyses allowing for the description of the changes in brain functional connectivity in aging for this specific cognitive ability. In doing so, it was expected that an evolution in functional connectivity pattern accompanying the semantic processing of words in aging would offer better insights on the driving forces of the neurofunctional reorganisation underlying these changes.

One of the studies that will be discussed in this talk concerns the relative preservation of receptive language abilities in older adults, which is associated with adaptive changes in cerebral activation patterns. These patterns have also been found to be task-load-dependent in some studies. In this functional magnetic resonance imaging study, data-driven spatial independent components analysis and hierarchical measures of integration were used to explore age-related changes in patterns of functional connectivity among cortical areas contributing to word fluency tasks in healthy young and older adults, as well as to assess the effect of age and task demands on the functional integration of a verbal fluency network. Results of this first study indicated that, despite similar high levels of performance during the verbal fluency conditions in both age groups, the functional integration of the speech production networks decreased with age, which however, was characterized by local changes modulated by task-demands within the verbal fluency network in high-performing older adults. These results suggest that age-related change in patterns of functional connectivity contributing to speech production are modulated by age and tasks demands. Given the noted diminution in level of integration, they also suggest that they reflect a life-long learning, or knowledge-building, process, which is compatible with similar tendencies noted in learning and in over-learning.

In a second study, we explore the neurofunctional patterns associated with another semantic ability in the same task, namely the ability to maintain a certain semantic category of word production, as well as the ability to switch from one semantic category to another. In addition, differences between individuals were explored by reference to their cognitive style characterize as executive abilities pattern. At this point, the results are essentially of a standard neurofunctional activation standpoint, but they indicate further characteristics of the reorganization pattern, adding a personal cognitive style dimension. Indeed, the pattern of neurofunctional activation was diametrically contrasted between the two older groups according to their cognitive executive profile. Functional connectivity results are still to come for that second study, but it is expected that they will shed new lights on the age-related neurofunctional reorganisation according to cognitive styles.

In conclusion, the series of studies discussed show the importance of functional connectivity as a measure which is more sensitive to the multidimensional nature of the neurofunctional reorganisation occurring in normal aging.

S28.3
NEURAL NETWORK CHANGES MEDIATING MOTOR SKILL LEARNING AND CONSOLIDATION

Julien Doyon
Unité de Neuroimagerie Fonctionnelle Université de Montréal Montréal, Canada

I will discuss the results of our functional connectivity data analyses on motor learning and consolidation using a functional integration approach (data driven connectivity method). The latter will provide evidence for the role of the cortico-striatal and cortico-cerebellar systems, together with the spinal cord, in learning novel motor sequences. Moreover, this work will show that motor memory trace consolidation is a sleep-dependent process, characterised by functional connectivity changes in the cortico-striatal system.

S28.4
EXECUTIVE CONTROL FUNCTIONAL NETWORKS IN BILINGUALS AND MONOLINGUALS

Ana Inés Ansaldo
Loboratoire de Plasticité cérébrale, Communication et Vieillissement, CRIUGM, Université de Montréal Perform Center, Concordia University, Montreal, Canada

Both the early and late acquisition of two or more languages have been related to an enhanced ability to exercise cognitive flexibility during multi-tasking. Learning two languages also provides building blocks for cognitive reserve and increases functional efficiency at older ages. The so-called bilingual advantage in interference control tasks has been studied with the Simon task, among others. The mixed evidence from the existing studies has led to contradictions in the literature regarding the bilingual advantage. Moreover, fMRI evidence on the neural basis of interference control mechanisms with the Simon task is limited. Previous work by our team showed that equivalent performance on the Simon task was associated with different activation maps in elderly bilinguals and monolinguals. Thus, unlike elderly monolinguals, who recruited frontal areas to resolve interference in the Simon task, elderly bilinguals recruited parietal areas – namely the left inferior parietal lobule – responsible for visuo-spatial processing. In other words, reliance on the right middle frontal gyrus, an area particularly vulnerable to healthy and pathological aging, was observed only in monolinguals. This represents a bilingual advantage at the neurofunctional level, given that bilinguals seem not to need to rely on brain areas that are more likely to be affected by aging. This might cause the bilingual brain to be better equipped to face cognitive aging. We have recently reanalyzed this data using graph theory, and particularly a Small World Approach to functional connectivity analysis to whole brain emerging networks.

An individually processed whole-brain functional connectivity analysis was performed on fMRI data collected during the Simon task on the elderly bilingual and monolingual participants recruited for our previous work, so as to characterize the topology of these networks. A node-by-node analysis led to the identification of the specific topology that characterized the bilingual and monolingual functional networks and the degree of connectivity between each node across groups.

Despite equivalent behavioral performance across groups, network connectivity associated with equivalent performance on the Simon task differed across bilinguals and monolinguals. Specifically, bilinguals showed greater connectivity in the inferior temporal sulcus, which plays a role in visuo-spatial processing, whereas in monolinguals, greater connectivity involved visual, motor, executive functions and interference control circuits.

Discussion and Conclusions: The respective networks for the monolingual and the bilingual groups showed identical properties at the global network level; however, network differences were observed across groups, at the node level in reference to these regions of interest. Specifically, the monolingual brain network included a larger set of connected areas than the bilingual group network. The evidence suggests that interference control tasks consume more neurofunctional resources in monolinguals, and that this is so even in the context of a nonverbal interference control task. Hence, the bilingual brain resolves visuo-spatial interference more economically than the monolingual brain, by allocating fewer and more clustered regions to the task. These results demonstrate a larger global efficiency in task performance in bilinguals as compared to monolinguals. Also, the provided evidence filters out the task-specific so-called bilingual advantage discussed in the literature, and posits the bilingual brain is more efficient than the monolingual brain in dealing with interference at a broader level. These findings contribute to enhancing our understanding of successful aging.

S28.5
REORGANIZATION OF LANGUAGE NETWORKS IN INDIVIDUALS WITH APHASIA

Olga Dragoy
Neurolinguistics Lab, National Research University Higher School of Economics, Russiaa

While resting state networks, which reflect the large-scale functional architecture of the human brain, have been extensively investigated in healthy population, similar studies remain less common in brain-damaged people and especially in people with aphasia. Specifically, the patterns and the role of language networks reorganization in aphasia have not been clarified yet. The aim of this study was to investigate differences in resting state language networks in people with aphasia and non-brain-damaged individuals.

Thirty two people with aphasia due to a stroke in the left hemisphere and 32 healthy age-matched individuals participated in the study; all were right-handed native speakers of Russian. T2*-weighted BOLD images (TR/TE/FA = 3 s/50 ms/90°; $3.9 \times 3.9 \times 3.75$ mm voxel; 64×64 matrix; 35 slices; 180 time points) and high-resolution T1-weighted images (TR/TE/FA = 1.9 s/2.93 ms/15°; $1 \times 1 \times 1$ mm voxel; 256×256 matrix; 176 slices) were acquired for each participant on a 1.5 T Siemens Magnetom Avanto scanner. Participants were instructed to stay relaxed in the scanner, with their eyes closed; no active task was given.

Data preprocessing was performed in SPM8. Following the procedure recommended by Allen et al., we identified resting state networks using group independent component analysis in GIFT. As a result, in a joint cohort of 64 participants, a number networks were revealed, including language, default mode, frontal executive, attentional, higher visual and visual functional networks. We used univariate tests corrected for multiple comparisons over all networks to test the difference between people with and without aphasia in the intensity of resting-state spatial maps, related to the connectivity and degree of co-activation within a network. The effect of group was only found significant in one language networks, which involved the bilateral temporal network (BTN), encompassing superior and middle temporal gyri bilaterally. To establish the direction of the group effect, using SPM8 we compared the intensity of activation between people with aphasia and healthy individuals in binary masks of the two (left and right) components of the BTN. The left part of the BTN displayed stronger intensity of spontaneous activity in healthy individuals than in people with aphasia, while the right part of the BTN was more strongly activated in people with aphasia. Specific parts of the right BTN correlated with the post onset time and the location of the lesion, but not with aphasia type, severity, nor with language scores.

To conclude, a clear asymmetry has been found between healthy individuals and brain-damaged people with aphasia, regarding a language network. The left-lateralized temporal component of that network was more strongly activated in healthy people, in contrast to the right temporal component, which was more strongly recruited in people with aphasia and modulated by the post onset time and the lesion location. More intense engagement of the right hemisphere language-related homologues under the condition of a left-hemisphere damage might underlie this asymmetry.

S29 Room: Marie Louise 2 SUBSTANCES OF ABUSE DURING PREGNANCY: IS IT ALL PLEASURE?

Chair: Carla Cannizzaro, IT

S29.1 MATERNAL Δ9-THC: FUNCTIONAL CONSEQUENCES ON OFFSPRING MESOLIMBIC DOPAMINE TRANSMISSION

Miriam Melis
Dept. Biomedical Sciences, Div. Neuroscience and Clinical Pharmacology, University of Cagliari, Italy

Marijuana is the most common illicit drug used by pregnant women. Clinical studies on long-term effects of marijuana smoking during pregnancy show its detrimental impact on cognitive and behavioral development of offspring from early childhood until later in life. Hence, early exposure to marijuana psychoactive ingredient, Δ9-tetrahydrocannabinol (THC), might induce enduring adaptations of brain reward dopamine (DA) system resulting in maladaptive behavior, ranging from affective dysregulation to addiction vulnerability. However, preclinical studies on the impact of marijuana use on development of reward DA pathway are surprisingly lacking.

To this aim, Sprague Dawley dams were administered THC (2 mg/kg s.c.) once per day from GD 5 to GD 20. All the experiments were performed during the third and fourth week after birth in both THC and vehicle offspring. Standard ex vivo whole cell patch clamp recordings were performed. Both basal and THC-induced extracellular DA levels were measured by brain microdialysis. Individual differences in basal and THC-induced locomotor and exploratory activity, risk-taking and gating functions were tested. Endocannabinoid levels were measured by using mass-spectrometry. Intermittent access alcohol two-bottle choice paradigm were performed. Here, we found that prenatal THC exposure induces a strengthening at afferent excitatory synapses on VTA DA neurons, which together with abnormal endocannabinoid system function, might produce a persistent excitatory drive of the DA pathway underlying an at-risk phenotype for psychosis and alcohol seeking during adolescence. Altogether, these results reveal molecular and synaptic changes that might help identifying molecular substrates and effective strategies for prevention and treatment of detrimental effects of prenatal THC exposure, which confer vulnerability towards discrete psychiatric conditions.

S29.2 LONG-TERM TRAJECTORIES IN ALCOHOL CONSUMPTION FROM THE MOTHER TO THE OFFSPRING: A "THIN RED LINE"

Carla Cannizzaro
Department of Sciences for Health Promotion and Child and Maternal care G. D'Alessandro, University of Palermo

Alcohol binge drinking is on the rise among young women and during pregnancy (1, 2) and this constitutes a major concern because of persistent neurobehavioral deficits in offspring, including increased vulnerability to substance abuse (3). This study modelled binge-like alcohol consumption in female rats and investigated alcohol drinking trajectories during pregestational time, pregnancy and lactation; in adulthood, offspring behavioural phenotype and alcohol vulnerabil-

ity were evaluated. Female rats underwent continuous- (CA) or binge-like intermittent (IA) two-bottle choice between water and 20% alcohol along 12 pre-gestational weeks and throughout gestation and lactation, and were assessed for maternal behaviour. In adulthood, offspring was tested for behavioural reactivity, anxiety- and depressive-like behaviour and cognitive functioning, along with alcohol vulnerability in a free-choice paradigm (10% alcohol and water). IA access augmented rats alcohol intake during pre-gestational time ($p < 0.001$) and lactation ($p = 0.001$), while reduced alcohol consumption during pregnancy ($p < 0.001$), with respect to the exposure to CA. Alcohol drinking habit disrupted spontaneous maternal behaviour more in IA- than in CA rats ($p < 0.001$). Perinatal alcohol exposure decreased offspring's behavioural reactivity in the open field ($p < 0.001$) and open arm preference in the elevated plus maze ($p < 0.01$), while increased immobility in the forced swim test ($p < 0.01$), when compared to controls; notably, perinatal IA-group displayed learning and memory impairment ($p < 0.05$) and decreased cognitive flexibility ($p < 0.5$) in the water maze with respect to controls. Perinatal IA alcohol offspring displayed a greater alcohol preference as baseline and after deprivation than perinatal CA rats ($p < 0.01$).

Our data indicate that alcohol drinking patterns induce discrete drinking trajectories during pre-gestational time, pregnancy and lactation in female rats. Moreover, long-lasting binge-like alcohol impairs mother-infant interaction and increases the occurrence of long-term detrimental consequences in the offspring.

S29.3
ENVIRONMENTAL TOBACCO SMOKE EXPOSURE IN THE EARLY POSTNATAL PERIOD

Marcourakis Tania
Department of Clinical and Toxicological Analysis, University. of Sao Paulo, São Paulo, Brazil

Brain development represents a period of vulnerability and several substances can induce neurotoxicity in this phase. Our aim is to study the effects of the environmental tobacco smoke (ETS) exposure during postnatal early brain development. Mice were exposed to a mixture of central and lateral tobacco smoke of the reference cigarettes 3R4F from the 3rd (P3) to the 14th (P14) day of life, twice a day (one hour each exposure, at 8 a.m. and 4 p.m.). It was evaluated learning and memory (Morris water maze), synaptogenesis markers (synapsin, synaptophysin and BDNF) in the hippocampus, as well as the myelination of nerve fibers in the optic nerve by morphometric analysis and the levels of Olig1 and MBP, markers in the cerebellum, diencephalon, telencephalon and brainstem during infancy (P15), adolescence (P35) and adulthood (P65). We also assessed mice glucose metabolism through PET/CT using the same exposure protocol. Our results showed that ETS induced impairment in learning and memory in all the ages evaluated. ETS also induced impairment in synaptic markers, by a decrease in synapsin, synaptophysin and BDNF in the hippocampus as compared to the control group. The percentage of myelinated fibers in the optic nerve in childhood and MBP levels in telencephalon and brainstem were lower in adolescence exposed to ETS compared to the control group. In cerebellum, there was an increase in MBP levels in infants and a decrease in adults compared to the control group. PET/CT analysis showed a decrease in glucose metabolism of mice exposed to ETS, in areas such as the limbic system. Taken all together, our results suggest that the exposure to ETS in early postnatal period induces impairment to the brain development. It is noteworthy that these effects are most evident during infancy, however not all effects are reversed in adolescence or even in adulthood.

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S29.4
IN-UTERO CANNABIS

Olivier Manzoni
INMED INSERM U901 Parc Scientifique de Luminy BP 13 13273 MARSEILLE Cedex 09, France

Although cannabis (hashish, marijuana) is the most commonly consumed/abused illegal drug by pregnant women, very little is known on the consequences of cannabinoid exposure during fetal neurodevelopment and its repercussions on synaptic and behavioral processes. Human studies converge to indicate psychiatric, cognitive, and behavioral effects of cannabis use in both adults and children and the progeny of women users. Thus, exposure to the principal psychoactive ingredient of cannabis, Δ-9THC induces profound physiological changes in the developing brain, which may lead to aberrant behavioral features in adults and specifically to a spectrum of protracted behavioral, neuronal and synaptic effects. These deleterious durable effects depend on age, gender, genetic, and/or environmental influences.

The cellular underpinnings of the pathological consequences of in-utero cannabis exposure (IUC) remain to be fully elucidated. Specifically, how IUC modifies synaptic connectivity in the circuits involved in emotional behavior and high cognitive function remains obscure.

Here we deciphered the consequences of chronic, intermittent and/or acute exposure to cannabis during fetal life on synaptic functions in identified brain circuits and on associated behaviors. Considering its integral role in motivated behaviors, social interaction and major diseases such as depression and addiction, we studied mesocorticolimbic circuits that include the ventral tegmental area, the nucleus accumbens, the amygdala and the pre-

frontal cortex. We used a multiple scale approach that combined *ex-vivo* electrophysiology and imaging, pharmacological manipulations and optogenetic control of disambiguated synapses, behaving analysis and a new multivariate analysis. Our data identify new synaptic maladaptation related to the profound social and cognitive impairments resulting from fetal cannabis exposure.

S30 Room: Reading Room SPROGRAMMING OF OFFSPRING DEVELOPMENT BY MATERNAL ENVIRONMENT

Chair: Muriel Koehl, FR

S30.1 EARLY NUTRITIONAL INTERVENTION PREVENTS THE EARLY-LIFE STRESS INDUCED COGNITIVE IMPAIRMENTS

Aniko Korosi

Swammerdam Institute for Life Sciences, Centre for Neuroscience, University of Amsterdam, Science Park 904,1098 XH, Amsterdam, The Netherlands

Early-life stress (ES) is associated with cognitive decline in adulthood. Mechanisms underlying this programming are unknown and the possible role of nutrition has been largely ignored. Essential nutrients such as methyl donors (MD; B-vitamins, methionine) and polyunsaturated fatty acids (PUFAs; omega-6 (n6) and n3) are key for brain development and epigenetic machinery, known to be involved in programming by ES. We study 1) if chronic ES affects these nutrient levels, 2) if early dietary intervention with MD or PUFAs prevents the ES-induced cognitive impairments and 3) mechanisms mediating the effects of these dietary interventions at hormonal, structural, and epigenetic level. We used a chronic ES mouse model consisting of exposing dams to limited nesting/bedding material from postnatal day (P) P2-P9, resulting in cognitive decline in adulthood. Dietary intervention consisted of subjecting mice to a methyl donor supplemented diet between P2-P9 or a diet with altered n6/n3 ratio resulting in high n3 levels between P2-P46. Chronic ES resulted in; i) reduced hippocampal methionine and increased n6 levels at P9, which is restored by early dietary intervention with MD and high n3, respectively. In addition, ii) high n3 diet prevented the ES-induced cognitive impairments as assessed by the object recognition (OR), object location (OL) and morris water maze (MWM) tasks, while the MD diet was able to prevent impairment in OR and MWM acquisition but did not affect OL performance, possibly due to the shorter duration of the MD intervention. And iii) high n3 diet prevented the reduction of adult born neuron survival caused by ES, suggesting that the beneficial effects of the diet may, at least partly, be mediated by preserving the neurogenic capacity in the ES-exposed offspring. Further characterization of the lasting effects of ES and these diets on nutritional status in peripheral and central organs is currently under investigation.

These results highlight for the first time the relevance of nutrients in the programming effects of ES and point to early dietary intervention as a novel non-invasive target to prevent the ES-induced deficiencies.

S30.2 DENIAL OF THE REWARD OF MATERNAL CONTACT AFFECTS SOCIAL BEHAVIOUR AND THE SEROTONERGIC SYSTEM IN THE ADULT RAT BRAIN

Fotini Stylianopoulou

Biology-Biochemistry lab, School of Health Sciences, National and Kapodistrian University of Athens, Athens, Greece

Early experiences, particularly mother-offspring interactions are key determinants of adult brain function and behavior. We have developed a model of early experiences in which rat pups, during postnatal days 10–13, are trained to learn to find their mother within a T-maze. Upon finding her, one group is allowed to enter the mother-containing cage and receive the expected reward of maternal contact (RER), while the other group is denied entrance, and the consequent expected reward (DER). The DER experience had long term effects resulting in reduced levels of oxytocin receptors (OTR) in the amygdala, probably as a consequence of increased methylation in the OTR promoter, which was detected at cytosines -11 and -65. Furthermore, adult DER males had decreased serotonergic neurotransmission in their brain. At the behavioral level, DER animals displayed deficits in sociability: During adolescence DER animals engaged more in aggressive-like play, and in adulthood displayed inappropriate, proactive, aggressive behavior in the resident-intruder test, while in the social interaction test they spent less time exploring their conspecific. In addition, they showed increased depressive-like behavior as assessed by increased immobility time in the forced swimming and anhedonia in the sucrose preference test. The DER experience in spite of its mild adversity, has significant long-term effects on emotional and social behavior and can be considered as a model of child neglect.

S30.3 PRENATAL STRESS AND PROGRAMMING OF STRESS-INDUCED EMOTIONAL AND MEMORY STATES

Muriel Koehl
Neurogenesis and Physiopathology group, INSERM U1215, Neurocentre Magendie, Bordeaux, France

Life events in childhood, experienced as early as during in utero life, play a pivotal role in determining and shaping adult behavior. In particular, we have previously shown that prenatal stress (PS) leads to a complex behavioral syndrome (i.e. deficits in spatial relational memory, emotional dysfunction, drug abuse) suggesting that early stressful events constitute a developmental risk factor for psychiatric disorders. Recently it has been proposed that an alteration in pattern separation (i.e. an inability to form different memories of similar episodes by generating distinct representations of the relationships comprising the events) contribute to overgeneralisation to aversive stimuli that characterize posttraumatic stress disorder (PTSD) patients.

Here we first analyzed - using a fear paradigm - the impact of PS on pattern separation. We found that the ability of PS mice to discriminate two similar contexts was impaired whereas their ability to discriminate two different contexts was preserved. Then we analyzed the memory profile of PS mice in a paradigm allowing to evidence PTSD-associated memory disturbances, i.e. hypermnesia for salient traumatic cues that do not necessarily predict the trauma, and amnesia for peritraumatic contextual cues. We found that PS mice exposed to a stressful event in adulthood develop such a PTSD-like memory. We further found that this paradoxical memory profile is associated with epigenetic changes within the hippocampal-amygdalar network.

Altogether, these results indicate that PS leads to pattern separation impairments and PTSD-associated memory disturbances without leading to an overgeneralization. It could thus constitute a useful model to identify vulnerability/resiliency factors for the development of psychiatric disorders.

S30.4
TRANSGENERATIONAL ACCUMULATION OF IMPAIRMENTS IN MATERNAL BEHAVIOUR FOLLOWING POSTNATAL SOCIAL STRESS

Chris Murgatroyd
Tufts University Cummings School of Veterinary Medicine, North Grafton, Massachusetts, USA

Early adversity such as depressed maternal care can have long-term physiological and behavioral effects on offspring and future generations. Exposure to chronic social stress (CSS), an ethologically model of postpartum depression and anxiety, during lactation impairs maternal care and exerts similar effects on the F1 dam offspring of the stressed F0 dams. These changes associate with increased corticosterone and neuroendocrine alterations. CSS F2 offspring further display decreased social behavior as juveniles and adults and decreased basal levels of corticosterone. We investigated the transgenerational inheritance of alterations in maternal behavior in F2 CSS dams together with neuroendocrine and immune markers to explore whether aspects of maternal behavior are transgenerationally inherited through immune and neuroendocrine mechanisms.

We found that maternal care behavior in the F2 dams is more severely impaired than in the F0 and F1 dams and the expression of maternal anxiety is expanded in F2 dams. This occurred together with reduced basal cortisol (in contrast to an increase in F1 dams), a lack of changes in neuroendocrine gene expression, and reduced serum ICAM-1 (intercellular adhesion molecule-1) levels - a marker for inflammation and blood-brain barrier integrity.

The results support the hypothesis that the effects of chronic social stress can accumulate across three generations to depress maternal care, increase maternal anxiety, and alter basal functioning of the immune system and hypothalamic pituitary adrenal axis.

S31 Room: Reading Room
A NOVEL WAY TO APPROACH NEUROPSYCHIATRIC DISORDERS: INVESTIGATING NEW MOLECULAR PATHWAYS

Chair: Luigia Trabace, IT

S31.1
SOLUBLE BETA AMYLOID: A BRIDGE BETWEEN ALZHEIMER'S DISEASE AND DEPRESSION?

Maria Grazia Morgese
Dept. of Clinical and Experimental Medicine, University of Foggia, Foggia, Italy

Recent evidences indicate that the prevalence of depression as well as Alzheimer's disease (AD) have reached epidemic proportions in last decades. Furthermore, depressive state has been pointed as an early manifestation of AD, advocating an overlap between these neuropathological events. In this regard, we have demonstrated that central soluble beta amyloid 1-42 (Aβ) peptide induces a depressive like-behavior in rats, with altered hypothalamic pituitary adrenal (HPA) axis activation, reduced cortical serotonin and neurotrophin levels. In searching for new risk factors for these pathologies, maternal malnutrition is emerging as a potential cause factor for developing mental illness in later adulthood, including depression. Therefore, we have studied the effect of lifelong exposure to diets differently en-

riched in n-3, n-6 (poor in n-3), as well as n-6/ n-3 polyunsaturated fatty acids (PUFA) balanced, on immobility time displayed on the forced swimming test (FST), along with neuroendocrine quantification of HPA axis parameters in offspring rats. Results showed that n-6 PUFA enriched diet increased depressive- and anxiety-like behaviors, as shown by the elevation in the immobility time in the FST test and self-grooming in the open field test. Those pro-depressive effects were accompanied by reduced cortical serotonin and enhanced plasmatic Aβ1-42 levels. Furthermore, plasmatic corticosterone and hypothalamic corticotropin releasing factor levels were significantly increased in animals fed with n-6 rich diet. Interestingly, chronic stress is considered a widely accepted risk factor for the development of both depressive symptoms and AD pathology. Indeed, high cortisol levels, and thus HPA axis hyperactivity, have been indicated as the most frequent alteration in patients affected by depression and AD. On the other hand, neuroinflammation represents a common link between depression and AD and the cyclooxygenase II (COX-2) enzyme seems to play a crucial role. Thus, in our study, we tested *in vivo* the effect of sub-chronic celecoxib, a selective COX-2 inhibitor, on the Aβ-induced model of depression and we associated *ex-vivo* quantification of monoamines and Aβ in order to evaluate a possible mechanism of action. We found that celecoxib prevented the increase in immobility and the decrease in swimming frequency, as well as the reduction in serotonin content at prefrontal cortex level induced by the peptide. In addition, an Aβ-lowering effect was also evidenced. Taken together our results indicate that Aβ levels can be considered as novel biomarker also for depression, and treatments or dietary interventions able to modify the peptide levels can be suitable therapeutic approaches for either AD or depression.

S31.2
DETRIMENTAL AND BENEFICIAL EFFECTS OF REACTIVE OXYGEN SPECIES IN PSYCHIATRIC DISORDERS: TOWARD NOVEL PHARMACOLOGICAL TARGETS

Stefania Schiavone

Department of Clinical and Experimental Medicine, University of Foggia, Foggia, Italy

Total reactive oxygen species (ROS) amount depends on the physiological activity of several biological systems, implicated both in their production (such as mitochondria and NADPH oxidases) or degradation (such as the glutathione system, the superoxide dismutases, as well as the non enzymatic ROS scavengers). A fine balance between ROS producer and degrading systems, also known as redox equilibrium, normally occurs in physiological conditions, thereby assuring the normal functioning of several biological processes. The Central Nervous System (CNS) is known to be particularly sensitive to possible redox equilibrium alterations, in terms of higher ROS production or reduced degradation. Indeed, in the CNS, ROS are key players of different physiological functions, going from regulation of neuronal fate to receptor signalling modulation. Emerging evidence has reported a crucial role of ROS in the pahogenesis of psychiatric disorders. In particular, we have demonstrated a crucial role of the NADPH oxidase NOX2-derived oxidative stress both in animal models of psychosis and in human psychiatric conditions. However, although the majority of the available published data mainly accounts for a detrimental role of free radicals in the pathogenesis of psychiatric disorders, new intriguing insights about a possible neuroprotective role of ROS in neurodegenerative and mental diseases development are arising. In particular, recent observations from our group, obtained from the ketamine perinatal mouse model of psychosis, suggest a possible neuroprotective role of the NADPH oxidase NOX1 enzyme against ketamine-induced excitoxicity and loss of GABAergic parvalbumin interneurons, probably via specific neurotrophin-associated molecular pathways. Thus, the identification of different roles of NOX enzymes in the pathogenesis of psychosis might provide new standpoints for the pharmacological targeting of this enzymatic system and identify novel therapeutic approaches for the treatment of these disorders.

S31.3
DEFICIT OF NEUROTROPHIN SIGNALING IN ALZHEIMER'S DISEASE: SEARCHING FOR NEW PHARMACOLOGICAL TARGETS

Filippo Caraci

Department of Drug Sciences, University of Catania, Catania, Italy; IRCSS Associazione Oasi Maria S.S., Institute for Research on Mental Retardation and Brain Aging, Troina, Italy

An impairment in the signaling of different neurotrophins such as Nerve Growth Factor (NGF) and Transforming-Growth-Factor-β1 (TGF-β1) has been demonstrated in Alzheimer's disease (AD) brain in an early phase of the disease. Deficit of TGF-β1 seems to be also a common pathophysiological event both in depression and AD. Depression is a risk factor for the development of AD and the presence of depressive symptoms significantly increases the conversion of Mild Cognitive Impairment (MCI) into AD. Interestingly, a continued long-term treatment with antidepressants reduces the risk to develop AD. Among antidepressants, selective reuptake inhibitors (SSRIs), such as fluoxetine and sertraline, increase circulating TGF-β1 levels which

are reduced in depressed patients. We assessed whether fluoxetine caters the potential to be protective against Aβ-induced neurodegeneration via a TGF-β1-mediated action.

We examined the neuroprotective activity of fluoxetine both in pure and mixed rat neuronal cultures challenged with synthetic Aβ(1–42) oligomers (1 μM).

Therapeutic concentrations (100 nM–1 μM) of fluoxetine significantly prevented Aβ-induced toxicity in mixed glia-neuronalcultures, but not in pure neuronal cultures, whereas serotonin did not mimic fluoxetine effects. Glia-conditioned medium collected from astrocytes challenged with fluoxetine protected pure cortical neurons against Aβ toxicity. The effect was lost in the presence of a neutralizing antibody against TGF-β1 in the conditioned medium, or when the specific inhibitor of type-1 TGF-β1 receptor, SB431542, was added to pure neuronal cultures. Accordingly, a 24 hr treatment of cortical astrocytes with fluoxetine promoted the release of active TGF-β1 in the culture media through the conversion of the latent form to the mature form of TGF-β1. Our data demonstrate that second-generation antidepressants are neuroprotective *in vitro* against Aß-induced neurodegeneration by rescuing TGF-β1 signaling and also suggest that drugs able to increase the release of active TGF-β1, such as fluoxetine, might represent new neuroprotective tools for the treatment of AD.

S31.4
INVESTIGATING IMMUNE-INFLAMMATORY TARGETS IN TRANSLATIONAL ANIMAL MODELS OF SCHIZOPHRENIA AND THEIR RESPONSE TO N-ACETYL CYSTEINE

Brian Harvey
Division of Pharmacology, and Center of Excellence for Pharmaceutical Research, School of Pharmacy, North West University, Potchefstroom, South Africa

Prenatal inflammation, early life adversity and drug abuse are risk factors for schizophrenia. Apart from altered cortico-striatal dopamine (DA), mitochondrial and immune-inflammatory-redox dysfunction are evident. Identifying new biological targets and novel treatments is critical. We studied the bio-behavioral effects of N-acetyl cysteine (NAC), a glutathione precursor, in maternal-immune activation (MIA), social isolation rearing (SIR) and methamphetamine-based dual-hit models of schizophrenia, where possible comparing it to clozapine (CLOZ) and CLOX+NAC. Post-weaning SIR animals received vehicle, NAC (150 mg/kg/day), CLOZ (5 mg/kg/day) or CLOZ+NAC for the last 14 days of rearing. Prenatal inflammation (lipopolysaccharide 100 mg/kg s.c.) plus MA, or post-natal SIR plus MA exposed rats received pre-pubertal or pubertal MA (0.2–6 mg/kg s.c. b.i.d.) and NAC (150 mg/kg s.c.) for 16 days. Social interaction (SI), recognition memory (RM), prepulse inhibition (PPI) and cortico-striatal DA, adenosine triphosphate (ATP) and immune-inflammatory-redox markers were variably assessed in these models. SIR induced deficits in SI, RM and PPI, simultaneously increased striatal but reduced cortical ATP and DA, disordered cytokine balance, and increased kynurenine-quinolinate metabolism and oxidative stress. CLOZ and CLOZ+NAC, less so NAC, reversed these changes. NAC augmented CLOZ. Chronic MA and SIR evoked similar psychosis-like behaviours with changes in cortical but not striatal DA, although MA+SIR were not additive. Similarly MIA and MIA+MA compromised SI, RM and PPI and regional brain monoamines, although no additive effect was seen. MIA and MIA+MA increased plasma and brain markers of oxidative stress, reduced IL-10 and increased TNF-α. These changes were variably reversed by NAC. SIR- and MIA- induced schizophrenia-like bio-behavioural changes are responsive to CLOZ or NAC, with CLOZ+NAC augmentative in the SIR model. MIA+MA associated changes were responsive to NAC. Dual hits paradigms engender compensatory mechanisms preventing an additive effect. NAC offers promise in schizophrenia and MA-associated psychosis as adjunctive treatment or as early intervention strategy.

S31.5
NOVEL APPROACHES TO LINK QUANTITATIVE BIOLOGY TO NEUROPSYCHIATRY

Martien Kas
Groningen Institute for Evolutionary Life Sciences, University of Groningen, the Netherlands

Current nosology for the diagnosis of neuropsychiatric disorders, such as Schizophrenia and Autism, separates each into non-overlapping diagnostic categories. This separation is not based on their underlying etiology but on convention based clustering of qualitative symptoms of the disorder. While these diagnostic categories are sufficient to provide the basis for general clinical management, they do not describe the underlying neurobiology that gives rise to individual symptoms. The ability to precisely link these symptoms to underlying neurobiology would not only facilitate the development of better treatments, it would also allow physicians to provide patients with a better understanding of the complexities and management of their illness. To realize this ambition, a paradigm shift is needed to raise awareness and to build an understanding of how neuropsychiatric diagnoses can be based on variation in quantitative biological parameters. However, the main difficulty in the construction of biologically valid diagnoses is the lack

of objective biomarkers. Moreover, the uncertain relationship between diagnosis and underlying etiology has created difficulties for etiological research and made the generation of appropriate disease models and development of targeted treatments very difficult. As etiological research progresses, there has been a rethinking of these diagnostic boundaries and their usefulness in treatment and classification of neuropsychiatric disorders. This is partly based on the notion that there is more etiological overlap between psychiatric and neurodegenerative disorders than previously thought, and that they may better be described as domains of cross-disorder-related traits (rather than separable categories) that can be translated to rodent models.

S32 Room: Carlson Suite
EMERGING VIEWS ON DOPAMINE SIGNALING AND WHAT CAN WE LEARN FROM STATE OF THE ART TECHNOLOGIES IN ANIMAL MODELS OF PD

Chair: Rosario Moratalla, SP

S32.1
IMPACT OF OPTOGENETICALLY DRIVEN STRIATAL PROJECTION NEURONS IN L-DOPA-INDUCED DYSKINESIAS

Ledia F. Hernandez
HM-CINAC – Hospital Universitario HM Puerta del Sur, San Pablo-CEU University, Madrid, Spain

Levodopa (LDOPA) is the dopaminergic replacement therapy that alleviates the motor complications caused by dopaminergic striatal denervation that occurs in Parkinson disease (PD). However, LDOPA induced dyskinesias (LIDs) are the main undesired effect reported after a prolonged treatment. I will present recent data showing that simultaneous optical activation of the direct and indirect striatal pathways causes abnormal movement generation bypassing the dopaminergic receptor. This abnormal evoked moments, that I named optodyskinesias, strongly resemble the LIDs obtained classically after LDOPA administration in a striatal dopamine depleted animal model. Additionally, a molecular marker that has been described related to LIDs (Fosb) is significantly increased in the dopamine depleted side. Moreover, no motor behavior was induced with the laser stimulation in animals that did not have the dopamine depletion. These results are a potential breakthrough in the understanding of mechanisms and pathways involved in this pathological condition.

S32.2
SYNAPTIC PLASTICITY CHANGES AFTER L-DOPA IN THE LESIONED STRIATUM

Rosario Moratalla
Instituto Cajal, CSIC, CIBERNED, Madrid, Spain

The synaptic organization of striatal medium-spiny neurons (MSNs) confers to dopamine a central role modulating glutamatergic-signaling from cortex and thalamus differentially in both output-pathways, striatonigral (D1-MSN) and striatopallidal (D2-MSN). The loss of dopamine fibers in Parkinson's disease as well as chronic L-DOPA that induced dyskinesia produce severe alterations in the functioning of corticostriatal synapses. However, the specific changes in both types of MSN underlying these alterations is still unclear. Using BAC-transgenic mice to identify striatal projection neurons, we demonstrate that spine-pruning caused by DA-depletion in Parkinson's disease affects mature spines similarly in D1- and D2-MSNs, enhancing the excitability of both striatal-pathways but reducing synaptic-strength selectively in D2-MSN. L-DOPA treatment restores spine density, synaptic-transmission and excitability to normal values selectively in D2-MSNs. However, chronic L-DOPA-treatment also modifies DR-sensitization, enhancing D1R-signaling but reducing D2R-mediated responses. Our findings indicate that L-DOPA-induced dyskinesia is associated with abnormal spine morphology, modified synaptic transmission and altered EPSP-spike coupling, with distinct effects in D1- and D2-MSNs. These alterations could contribute to the loss of bidirectional synaptic-plasticity observed in dyskinesia.

S32.3
NON-MOTOR SYMPTOMS IN PARKINSON'S DISEASE: MODELING AND MECHANISMS

Gilberto Fisone
Department of Neuroscience, Karolinska Institute, Stockholm, Sweden

The use of animal models in the study of Parkinson's disease (PD) has led to the identification of numerous abnormalities affecting dopamine transmission and potentially linked to the development and manifestation of L-DOPA-induced dyskinesia (LID). One critical change associated with the depletion of dopamine occurring in PD is the development of sensitized transmission at dopamine D1 receptors (D1Rs). This phenomenon confers to L-DOPA the ability to activate a number of signaling pathways implicated in the short- and long-term regulation of striatal medium spiny neurons (MSNs),

and ultimately responsible for the emergence of LID. Here, we focus on recent findings on the effects of L-DOPA on chromatin modifications and transcriptional activators. Using a mouse model of PD, we found that L-DOPA, via activation of D1Rs, increases the phosphorylation of histone H3 on serine 28 (S28p), in a large population of striatal MSNs. Importantly, S28p occurs on genomic regions marked by trymethylation of lysine 27 (K27me3), which is catalysed by repressive proteins belonging to the Polycomb group (PcG) family. S28 phosphorylation of H3K27me3 leads to displacement of PcG proteins from the regulatory regions of genes coding for transcription factors involved in synaptic plasticity, such as Npas4 and Atf3. In line with these findings we found enhanced levels of both Npas4 and Atf3 in the striata of dyskinetic mice, suggesting the potential role of these proteins in the development of LID. In other studies we investigated the regulation of the c-Jun N-terminal kinases (JNK), which have been involved in various neuronal responses, including synaptic plasticity. Administration of L-DOPA results in a large increase in the phosphorylation/activation of JNKs and of its downstream target, cJun. These effects are produced in striatal MSNs and require D1R-mediated activation of the cAMP/DARPP-32 signaling cascade. The activation of JNK is accompanied by enrichment of phosphorylated c-Jun at the promoter of fosB, which codes for a transcription factor implicated in dyskinesia. The aberrant regulation of the JNK/c-Jun signaling cascade represents a novel target involved in the action of L-DOPA and potentially implicated in the long-term maladaptive changes of transcription implicated in LID.

S32.4
INTEGRATED DOPAMINERGIC AND SEROTONERGIC SIGNALING AT GLUTAMATERGIC STRIATAL SYNAPSES

Raffaella Tonini
Neuroscience and Brain Technologies Department, Istituto Italiano di Tecnologia, Genova, Italy

The dorsal striatum (DS) of the basal ganglia (BG) plays a critical role in voluntary movement, learning and motivation, and represents the primary site of dysfunction in psychomotor disorders. The DS receives glutamatergic cortical and thalamic afferents, which are extensively modulated by dopaminergic and serotonergic inputs. In the presence of behaviorally relevant stimuli, dopamine is assumed to reinforce motor actions through the potentiation of corticostriatal synapses. In contrast, the synaptic role of serotonin (5-HT) at striatal circuits is still unclear. In this study, we investigated the effect of manipulating serotonergic signaling on associative synaptic plasticity in striatal projection neurons of the direct pathway. By combining chemogenetic, optogenetic, and pharmacological approaches, we identified a novel synaptic mechanism underlying the input-specific neuromodulatory impact of decreased 5-HT-mediated signaling at glutamatergic striatal synapses.

S33 Room: Ballroom
DEVELOPING NOVEL TOOLS FOR ENDOCANNABINOID RESEARCH

Chairs: Mauro Maccarrone, IT and Stefanie Butini, IT

S33.1
A MODERN VIEW OF THE ENDOCANNABINOID SYSTEM AND NEED FOR SELECTIVE DRUGS

Mauro Maccarrone
Department of Medicine, Campus Bio-Medico University of Rome, Via Alvaro del Portillo 21, 00128 Rome, Italy. European Center for Brain Research/Santa Lucia Foundation IRCCS, Via del Fosso di Fiorano 64, 00143 Rome, Italy

Endocannabinoids (eCBs) are endogenous lipids able to activate cannabinoid receptors, the primary molecular targets of the cannabis (Cannabis sativa) active principle Δ9-tetrahydrocannabinol. During the last twenty years, several N-acylethanolamines and acylesters have been shown to act as eCBs, and a complex array of receptors, metabolic enzymes, transmembrane and intracellular transporters (that altogether form the so-called "eCB system") has been shown to finely tune their manifold biological activities. It appears now urgent to develop selective drugs that allow to dissect the contribution of the distinct components of the eCB system to the overall biological activity of these compounds, thus putting in a better perspective the relevance of its various elements as key-player of disease conditions. A modern view of the eCB system is presented here, and forms the basis for more rationale and effective therapeutic strategies to combat eCB-related human pathologies.

S33.2
NEW SELECTIVE DRUGS FOR DAG LIPASES

Mario van der Stelt
Department of Molecular Physiology, Leiden Institute of Chemistry, Leiden University, 2333 CC, Leiden, The Netherlands

2-Arachidonoylglycerol (2-AG) is an endocannabinoid that activates the cannabinoid receptors type 1 and 2. It also serves as an important lipid precursor for the eicosanoid signaling pathway. Consequently, 2-AG is involved in many physiological functions, including anxiety, food intake, inflammation, memory, pain sensation

and neurotransmission. Diacylglycerol lipases (DAGLs) are the main biosynthetic enzymes for 2-AG and their role in several pathophysiological conditions is currently under investigation. In this presentation I will discuss currently available DAGL inhibitors and their effects in preclinical models of neurodegeneration and metabolic disorders.

The emphasis will be on the development of centrally active DAGL inhibitors DO34 and DH376 and a structurally related control probe and their use, in combination with chemical proteomics and lipidomics, to determine the impact of acute DAGL blockade on brain lipid networks in mice [1]. Within 2 h, DAGL inhibition produced a striking reorganization of bioactive lipids, including elevations in DAGs and reductions in endocannabinoids and eicosanoids. We also found that DAGLα is a short half-life protein, and the inactivation of DAGLs disrupts cannabinoid receptor-dependent synaptic plasticity and impairs neuroinflammatory responses, including lipopolysaccharide-induced anapyrexia. These findings illuminate the highly interconnected and dynamic nature of lipid signaling pathways in the brain and the central role that DAGL enzymes play in regulating this network.

S33.3
NEW SELECTIVE DRUGS FOR CANNABINOID RECEPTORS

Uwe Grether
Roche Pharma Research & Early Development, Roche Innovation Center Basel, F. Hoffmann-La Roche Ltd., 4070 Basel, Switzerland

Despite the fact of several years of intense cannabinoid receptor 2 (CB2) research, still many fundamental questions regarding target location, exact mechanism of action and receptor trafficking remain unanswered. Particularly, the inducible nature of the receptor and the lack of highly specific antibodies have posed considerable obstacles in adressing these questions. Thus, novel, highly selective small molecule CB2 probes such as e.g. fluorescence- and radio- labelled ligands as well as covalent binders might help to answer some of the questions.

2,5,6-Trisubstituted pyridines/pyrazines [1] and triazolopyrimidines [2] were found to be novel, highly potent and selective CB2 ligands. Both series offer three independent exit vectors which have been used for exploring the introduction of different types of labels.

Details on the way towards novel CB2 specific radio- and fluorescence-labels, covalent binders, Raman probes as well as bifunctional probes will be the subject of this communication. We will report results on: i) The structure activity relationship with regard to human CB2 and CB1 binding and functional activity; ii) Mouse CB2 binding and functional data; iii) Early absorption, distribution, metabolism and excretion (ADME) properties of advanced probes including e.g. solubility, passive membrane permeation and lipophilicity data; and iv) First applications of these novel CB2 probes.

S33.4
DEVELOPMENT OF NOVEL ENZYME INHIBITORS OF THE ENDOCANNABINOIDS' CATABOLISM FOR THE TREATMENT OF EPILEPSY AND MULTIPLE SCLEROSIS

Stefania Butini
European Research Centre for Drug Discovery and Development (NatSynDrugs), University of Siena, Via Aldo Moro 2, 53100 Siena, Italy

The main endocannabinoids (ECs) anandamide (AEA) and 2-arachidonoylglycerol (2-AG), by stimulating cannabinoid CB1 and CB2 receptors (CB1R and CB2R), regulate relevant signalling pathways. The key enzymes involved in ECs catabolism are fatty acid amide hydrolase (FAAH) and monoacylglycerol lipase (MAGL). Their unique role in terminating ECs signaling and regulating the intracellular levels of AEA, 2-AG and other ECs supports their potential as therapeutic targets. Selective inactivation of ECs degrading enzymes represents an attractive approach for eliciting the desirable effects of CBR activation, while avoiding the negative (psychotropic, among others) effects of CB1R stimulation.

Epilepsy is the second most common neurological disorder with an incidence rate of 0.3–0.5% worldwide. Most of conventional anti-epileptic drugs have narrow therapeutic margin and require therapeutic drug monitoring. Despite the introduction of many II-generation anti-epileptic drugs, pharmacoresistant epilepsy has not been significantly reduced. A reduction of AEA in patients affected by temporal lobe epilepsy (TLE) has been clearly documented. Similarly, the neuroprotective role of AEA was confirmed by the kainic acid induced increase of AEA in hippocampus, which, without affecting 2-AG, provides "on demand" protection against acute excitotoxicity. Seizure activity at the cellular level initiates significant influx of calcium. Over time, impaired neuronal calcium homeostasis increases the activity of pro-oxidant cellular systems.The enocannabinoid system can delay or prevent excitotoxic damage by rebalancing the main excitatory/inhibitory systems and directly modulating mitochondrial function, thus preventing oxidative stress–related epileptogenesis. This rationale led to the proposal of FAAH inhibitors as therapeutic option for the prevention of epileptic disorders. To strengthen this hypothesis we demonstrated that NF1245 our potent and selective FAAH inhibitor (Ki = 160 pM), ameliorates the acute epileptic behavior and prevents hippocampal oxidative damage in rat model

of pilocarpine-induced epilepsy tested at 10 mg/kg. The same dose was also effective in TLE generated by electric kindling. Multiple sclerosis (MS) is a chronic, inflammatory autoimmune disease characterized by nerve demyelination that affects up to 2.5 million people worldwide. At present there is no drug available for controlling the disease progression of patients with progressive forms of MS, and no means to repair injured axons or protect neurons from further damage. In the past few years, an increasing body of evidence has suggested that ECs may have beneficial effects on the symptoms of MS and ECs have been suggested to be neuroprotective in this context. Therefore MAGL appears as a promising and reliable target for the treatment of MS. We have recently characterized a new class of beta-lactam-based MAGL inhibitors typified by NF1819.5 NF1819 is a highly potent and selective MAGL inhibitor, it increases the levels of 2-AG by blocking its degradation, thus acting as CBRs' indirect agonist. NF1819 was effective in reducing the clinical scores in mice suffering from experimental autoimmune encephalomyelitis and in limiting the associated demyelination and inflammation in the spinal cord in mice at the acute stage of the disease. These positive effects were prevented by co-administration of CB1R or CB2R antagonists, strongly suggesting the involvement of these receptors. These observations allowed us to define NF1819 as prototypic of a new class of innovative pharmacological tools for the management of MS progression.

S33.5
NEW SELECTIVE DRUGS FOR ENDOCANNABINOID TRANSPORT

Jürg Gertsch
Institute of Biochemistry and Molecular Medicine, National Centre of Competence in Research NCCR TransCure, University of Bern, Switzerland

Endocannabinoids are key lipid signals which roles are involved in neuronal, metabolic and inflammatory processes. While probes are available for most of the known proteins within the endocannabinoid system, endocannabinoid membrane transport remains poorly understood. The extracellular effects of the endocannabinoids anandamide and 2-arachidonoyl glycerol are terminated by enzymatic hydrolysis after crossing cellular membranes by facilitated diffusion. The lack of potent and specific inhibitors for endocannabinoid transport has prevented the molecular characterization of this process, thus hindering its biochemical investigation and pharmacological exploitation. Recent advances show that it is possible to potently and selectively inhibit endocannabinoid cellular reuptake, independent of the metabolic enzymes. Both potent inhibitors and tailored diazirin-containing photoaffinity probes provide new insights into the existence of a putative membrane target mediating endocannabinoid cellular trafficking in certain neurons and immune cells, thus uncovering a new pharmacological modulation.

S34 Room: Marie Louise 2
NOVEL PERSPECTIVES IN VISUO-MOTOR LEARNING AND OBJECT REPRESENTATION FOR ACTION AND PERCEPTION

Chairs: Annalisa Bosco, IT and Luca Turella, IT

S34.1
NEURAL NETWORKS UNDERLYING LEARNING OF MOTOR SKILLS FROM OTHERS

Roy Mukamel
School of Psychological Sciences and Sagol School of Neuroscience, Tel-Aviv University, Tel-Aviv, Israel

Visual input plays a significant role in performance and learning of motor skills. At the behavioral level, it has been shown that concurrent observation of actions performed by others can facilitate or interfere with ongoing executed actions depending on the congruency level between the two. Additionally, a higher tendency to implicitly imitate observed actions during social interactions has been reported (the Chameleon Effect). At the physiological level, action perception has been shown to evoke significant neural activity in circuits of the motor pathway (the Mirror Neuron System). This system has been suggested as a potential neurophysiological substrate for the behavioral phenomena described above.

During my talk, I will demonstrate that visual input of actions that are non-consciously perceived, still elicits significant neural activity in the mirror neuron system. Additionally, I will show that consciously perceived actions elicit implicit subsequent changes in behavior which can be predicted from evoked neural activity during observation. Moreover, we see differential behavioral and neural effects during action observation for the explicit purpose of learning that depend on the identity (right/left) and size of observed hand. Finally, I will describe a novel virtual-reality setup we developed in the lab which yields efficient performance gains in a limb that is not physically engaged in the training process. Together, these results have implications for efficient acquisition of motor skills and rehabilitation of patients with hemiparesis and shed light on the neurophysiology underlying behavioral phenomena such as the Chameleon effect.

S34.2
NEURAL CORRELATES OF OBJECT ENCODING FOR GOAL-RELATED ACTIONS

Luca Turella
Center for Mind/Brain Science (CIMeC) – University of Trento, Italy

In everyday life, we continuously interact with objects within the environment. To properly interact with them, we need to transform incoming sensory information into goal-directed motor outputs. Whereas our ability to plan an appropriate object-directed action based on sensory information appears effortless and simple, the underlying brain dynamics are still largely unknown.

Previous monkey neurophysiological studies demonstrated a wide set of regions within the fronto-parietal cortices representing object properties and their spatial location during action planning, but if this information is similarly encoded within the human brain is still an open question.

Here, we aimed at understanding how the human brain represents objects and their spatial location during the planning of goal-directed actions. In details, we asked participants to reach specific spatial positions within the surrounding space while recording their brain activity. Adopting a combination of univariate and multivariate analyses of neuroimaging data, we investigated how planned object-directed reaching movements were encoded. Cross-decoding multivariate analysis showed that these movements were encoded irrespective of specific properties of the target object even before the action was executed.

Our data widen previous investigations on motor control by demonstrating that actions are represented within the brain at a more "abstract" level, irrespective of specific properties of the target-object. These results open the path towards understanding the different contribution of these types of encoding during action planning. The possibility of representing objects and movements at a higher level of abstraction might be at the basis of the great flexibility of our daily interactions with the environment.

S34.3
THE INFLUENCE OF DIFFERENT PREDICTIVE CONDITIONS OF SUBSEQUENT ACTION ON OBJECT SIZE PERCEPTION

Annalisa Bosco
Dept. of Pharmacy and Biotechnology, University of Bologna, Italy

Humans frequently estimate the size of objects to grasp them. Researchers have concentrated their attention mainly on the grip aperture profile during the execution of grasping which is strongly correlated with object size and occurs well before the fingers come into contact with the object. The preshaping of the hand is a highly stereotyped motor pattern that is largely predetermined by object-related visual input. When an action is just intended but not executed yet, our perception is focused towards object visual properties that enable us to execute the action successfully. For example the orientation perception is enhanced during preparation of grasping action compared with a pointing for which object orientation is not important. This "enhanced perception" is triggered by the intention to grasp and is important to examine objects with the maximum possible accuracy. To study the effects of action execution on visual perception of object features, the saccadic adaptation is a phenomenon extensively studied for this purpose. In fact, we recently showed that inducing a saccadic adaptation to targets that systematically change their size during the saccade execution affects the visual perception of the target size. But whether there is ample evidence for visual perception changes in the oculomotor system, little is known about the perceptual changes induced by hand movements. Different studies about sensorimotor integration pointed to define a model that combines afferent sensory cues with previous knowledge about object features to estimate object properties and issue appropriate motor responses.The size-weight illusion represents a way to study the integration of action and perception induced by motor experience. The SWI arises when an individual lifts two objects of equal weight, but of different size, and perceives the smaller object as heavier. The SWI is believed to be a primarily haptic phenomenon and significantly decreases with action repetition. The SWI paradigm has been extensively studied, but no study has investigated how the brain estimates object size immediately after different types of hand movements without haptic feedback and the use of visual or weight illusions as well. Here, we aimed to study a feature-specific perceptual modulation after a reaching and a grasping action in different contexts.

Human participants were instructed to either grasp or reach to different sized bars without haptic feedback and, before and after the action, to perform a size perceptual task using a manual and a verbal report. Subjects performed estimation when they did not know the subsequent type of movement and when they were aware about the successive type of movement. The quantification of perception modification after action showed significant reduction of perceived size after the grasping movement compared to that after the reaching. Interestingly, the only knowledge of subsequent action type significantly affected the size perception before the movement execution with consistent results in both manual and verbal reports. This modification of perceptual re-

sponses observed here was consistent with the view that perceivers scaled the extents of graspable objects using the visual and proprioceptive feedback coming from the grip aperture during the movement. Such mechanism can be clearly described by the Bayesian model where action provides the likelihood and this latter is integrated with the expected size (prior). Beyond the action-modulation effect, the knowledge of subsequent action type influences the object perception. In fact, the defined or undefined context generates different perceptual responses independently from the modality used to answer.

S34.4
CORRELATIVE STUDY OF IMPAIRMENT OF LEARNING PROCESS USING VISUO-MOTOR ASSOCIATION AND MRI DATA IN PARKINSON PATIENT WITHOUT COMORBIDITIES

Boujraf Saïd
Clinical Neuroscience Laboratory, Faculty of Medicine and Pharmacy, Univ. of Fez, Morocco. Depart. of Biophysics & Clinical MRI Methods, Faculty of Medicine and Pharmacy, Univ. of Fez, Morocco

Parkinson's disease (PD) affects not only motor behavior, but cognitive and emotional functions as well. However, it is not clear yet whether cognitive and motor deficits relate to brain functional reorganization following the loss of midbrain dopamine neurons. In this study, we sought to investigate the functional networks activated during visuomotor learning in PD patients using BOLD-fMRI.

Fifteen patients were recruited for this study. They were selected on the basis of absence of any additional comorbidity. All patients were tested on a visuomotor learning task typical for activation the fronto-basal ganglia system, and underwent BOLD-fMRI and anatomical MRI using both motor and emotional paradigms. The fMRI data was processed using SPM12 package.

Analysis of behavioral data showed that learning abilities are impaired in PD patients compared to age-matched controls. Analysis of BOLD signal showed that brain activations have a lower dynamics during learning. The results still need further confirmation, especially in terms of functional connectivity analysis. This talk will address the potential mechanisms, especially in terms of functional connectivity within the fronto-striatal loops in PD patients as compared to controls.

S35 Room: Marie Louise 1
SENSORIMOTOR ACTIVITY ASSESSMENT IN HEALTHY AND PATHOLOGICAL CONDITIONS

Chairs: Kenneth P. Camilleri, MT, Tracey Camilleri MT, Owen Falzon, MT, Pierto Avanzini (IT) and Maddalena Fabbri Destro (IT)

S35.1
BEHAVIOURAL AND NEURAL CORRELATES OF SOMATOSENSORY DEFICITS IN ARM AND HAND POST STROKE

Geert Verheyden
KU Leuven - University of Leuven, Department of Rehabilitation Sciences, Leuven, Belgium

Somatosensation is sensory information arising from the skin, muscles and joints such as tactile, proprioceptive or discriminative stimuli. It is well known that somatosensory impairments negatively affect recovery post stroke; however, little is known about the extent of somatosensory impairments in the upper limb, their association with motor function and the neural correlates. Therefore, a cross-sectional, longitudinal and brain imaging study was conducted including 122, 32 and 38 patients, respectively. Results from the cross-sectional study showed that upper limb somatosensory impairments were common within the first six months post stroke, with prevalence rates ranging from 21–54%. Low to moderate associations were found between somatosensory and motor deficits (r = 0.22–0.61). Additionally, patients with visuo-spatial neglect had significantly more often and more severe somatosensory deficits, and associations between somatosensory and motor function were stronger compared to patients without neglect. Results from the longitudinal study showed that 41–63% of the patients experienced a somatosensory deficit within the first week post stroke and 3–50% at six months. In the acute phase, there were only very low associations (r = 0.03–0.20) between somatosensory and motor impairments, whereas at six months, low to moderate associations (r = 0.32–0.69) were found. In the brain imaging study, voxel-based lesion-symptom mapping was performed to investigate lesion contribution to somatosensory impairments in the upper limb. Results showed two core brain regions with a significant association to somatosensory deficits: the central parietal white matter, also referred to as the sensory component of the superior thalamic radiation, and the parietal operculum close to the insular cortex, representing the secondary somatosensory cortex. In conclusion, these studies showed that somatosensory impairments are common and suggests that the association with upper limb motor performance increases with time after stroke. Finally, stroke lesions in the sensory fibers of the superior thalamocortical radiation and parietal operculum are significantly associated with somatosensory impairments.

S35.2
NEUROREHABILITATION TECHNOLOGIES FOR STROKE

Andrei Agius Anastasi
Centre for Biomedical Cybernetics, University of Malta, Malta

Stroke is a leading cause of disability in adulthood today. It is estimated that 15 million people suffer from a stroke worldwide per year, a third of these remaining with permanent disability. Rehabilitation, through physiotherapy and other physical training, remains the gold standard for improving the quality of life of affected individuals and reducing disability. Nevertheless, with the massive technological advances seen in the last few decades, new ideas for stroke care and rehabilitation have been on an exponential rise. Neurorehabilitation research is at the forefront of robotics, brain computer interface, virtual reality and other cutting-edge technological fields throughout the world. The multidisciplinary cooperation within the field of biomedical cybernetics, between medicine and engineering, has allowed for great advances in either fields; research in cybernetic solutions have opened up new knowledge that has been invaluable for both sides, helping each sector advance. Furthermore, despite being very novel and scarcely validated, these upcoming solutions offer hope to person's with disability that may otherwise never regain their function and independence back. The number of randomized controlled trials and meta-analysis on the true effectiveness of such technology- and robot-based neurorehabilitation is now on the rise, with promising results. In this talk we will deal with the general concepts behind neurorehabilitation and physiotherapy goals and then move onto innovative, technology-based solutions that are currently being developed. In particular the use of EMG-based biofeedback systems, functional electrical stimulation, robotic end-effectors and exoskeletons, brain-computer interface, kinematics and virtual reality based rehabilitation platforms will be introduce. The concept of exergaming will also be entertained where the vision is that in the near future, such technology-based rehabilitation tools and games become common household gadgets.

S35.3
EXPLORING CORTICAL ACTIVITY MEASURED BY ELECTROENCEPHALOGRAPHY OF UPPER LIMB SENSORIMOTOR IMPAIRMENTS AND RECOVERY AFTER STROKE

Lisa Tedesco Triccas
KU Leuven – University of Leuven, Department of Rehabilitation Sciences, Leuven, Belgium; University of Malta, Department of Systems and Control Engineering, Malta

At six months post-stroke, 33–66% do not present with full recovery of upper limb function1. Identified predictors for poor upper limb sensorimotor recovery are increased stroke severity, more severe somatosensory and motor impairments and the presence of visuospatial neglect2. Somatosensory deficits are experienced by 21–54% of stroke survivors and negatively impact on upper limb use, reaching, grasping and dexterity. One of the methods of measuring cortical neurophysiological mechanisms of brain recovery in stroke is using electroencephalography (EEG). Disruption of neural connectivity can be measured by Event Related Desynchronisation (ERD) using EEG. Decrease in ERD of alpha and beta-bands oscillations of the affected sensorimotor areas compared to contralesional areas have been reported at rest and during movement of people with stroke with motor impairments 3,4. Currently, there is a gap in knowledge about the understanding of the recovery of underlying cortical activity of people with upper limb somatosensory and motor impairments during the different stages of stroke. Therefore, the aim of this review is to explore the current evidence about the changes of cortical activity measured by EEG in association with sensorimotor upper limb impairments in the acute, subacute and chronic stage after stroke. Electronic searches were conducted to identify the relevant studies. A total of 1607 unique papers were listed from all the databases. Results were independently reviewed by two reviewers and 617 papers were retained for further review after the eligibility assessment based on title. Abstract selection will be carried out during the next stage of the research which will be followed by full text review of the selected papers, risk of bias assessment and data collection and analysis. The final results of the review will be presented at the Symposium.

S35.4
THE RECRUITMENT OF SENSORIMOTOR SYSTEM DURING ACTION EXECUTION AND OBSERVATION AS REVEALED BY ELECTROPHYSIOLOGICAL INVESTIGATIONS

Pietro Avanzini
Istituto di Neuroscienze, CNR, Italy

Since the discovery of mirror mechanism, the motor system was recognized to have a double-life, playing a crucial role not only during action execution, but also while observing and recognizing the action performed by other individuals. In the first part of this talk, I will describe the key contribution that electrophysiological (mostly EEG) studies gave to the characterization of mirror mechanism in humans. Indeed, starting from the pioneeristic observations by Gastaut and Bert, through quantitative EEG and combined EEG-fMRI

results, electrophysiological studies allowed to describe the characteristics of mu rhythm, the cortical regions from which it originates, and the different reactivity that it shows in response to action execution, action observation and, more generally, motor-related stimuli. In the second part of the talk, I will show how a precise characterization of mu rhythm cortical generators may be achieved through a combined EEG and intracranial recording study.

S35.5
THE TIGHT LINK BETWEEN ACTION EXECUTION AND RECOGNITION IN CHILDREN WITH AUTISM: ECHOING AN OUT-OF-TUNE CHORD?

Maddalena Fabbri Destro
Istituto di Neuroscienze, CNR, Italy

Beside a huge amount of literature usually referring to the core social and communicative deficits in ASD, growing evidence have shown how ASD developmental profile is also characterized by movement impairments, affecting basic motor control, skilled motor gesture execution, praxis performance, and future actions planning. In addition, given the key role that motor system plays in social and cognitive functions, an impairment in the organization of ASD motor behavior may correlate with the developmental milestones and could be at the basis of typical socio-cognitive impairments.

Starting from these premises, in this talk I will present data collected in a behavioral study on praxis performance in both ASD and typically developing children. First, results on TD children not only show the developmental trajectory of praxic abilities, but also their concurrent relationship with action recognition skills. Subsequently, by comparing ASD with a matched TD subpopulation, we identify specific features of praxic organization that are impaired for ASD children both during action execution, and during recognition of actions. Finally, I report how these specific indices correlate with the ASD symptoms severity.

Taken together, all these results 1) reinforce the link between action execution and recognition, 2) suggest that a motor system deficit may be involved in autistic syndrome, being manifest both during action execution and observation, and possibly contributing to the core socio-communicative symptoms of autism.

S36 Room: Clermont Suite
THE MIND IN THE MATRIX: EXTRACELLULAR MATRIX MOLECULES AS CRITICAL FACTORS FOR PSYCHIATRIC DISORDERS

Chair: Constanze Seidenbecher, DE

S36.1
CAUGHT IN THE WEB OF DEPRESSION

Sabine Spijker
Department of Molecular and Cellular Neuroscience, Center for Neurogenomics and Cognitive Research, Neuroscience Campus Amsterdam, The Netherlands

Major depressive disorder (MDD) is a complex neuropsychiatric disorder that is characterized by persistent negative mood, a multifaceted anhedonic state, and impaired cognitive function. Here, we modeled sustained depression-associated memory deficits using the social defeat-induced persistent stress (SDPS) paradigm in rats. Importantly, these hippocampus-dependent cognitive deficits persist up to three months following a 5-day social defeat in rats. This state is characterized by increased levels of extracellular matrix (ECM) proteins, which resides in perisynaptic places, number of perineuronal nets (PNNs), reduced maintenance of hippocampus CA1 long-term potentiation (LTP), and reduced inhibitory input to CA1 pyramidal cells. Both the tricyclic antidepressant imipramine, and enriched housing as behavioral therapy, normalized expression of ECM/PNN proteins, and rescued the hippocampal LTP deficit and cognitive impairments. Furthermore, we found that these proteins crucially limit synaptic plasticity and also underlie cognitive impairments in our model, as *in vivo* breakdown of CSPG-rich PNN proteins in the CA1 region months after social defeat restored PNN levels, LTP, inhibitory input and memory function. We propose a novel mechanism by which PNN-mediated hippocampal cognitive deficits develop during a state of enduring depression, and identify a new therapeutic entry point to overcome these.

S36.2
NOT ONLY NEURONS: EXTRACELLULAR MATRIX ABNORMALITIES IN MAJOR PSYCHOSES

Sabina Berretta
Translational Neuroscience Laboratory, Mclean Hospital, Belmont, MA; Dept. of Psychiatry, Harvard Medical School, Boston, MA; Program in Neuroscience, Harvard Medical School, Boston, MA, USA

The long-held view of psychiatric disorders as 'disorders of the neurons' has been challenged in recent times by evidence supporting the involvement of glial cell and extracellular matrix (ECM) in the pathophysiology of these disorders. Such evidence represents a significant departure from mainstream 'neuron-centric' views, bringing to the forefront the idea that interactions between neurons, glia and ECM may play a key role in the pathophysiology of psychiatric disorders. Support

for key roles of perineuronal nets (PNNs) and ECM molecules in regulating synaptic functions and plasticity is accumulating in parallel with findings of decreased PNNs in subjects with schizophrenia and bipolar disorder. PNNs are specialized perisynaptic aggregates of ECM molecules ensheating distinct neuronal populations. Glial cells have been shown to play a crucial role in PNN formation, providing key molecular components, such as chondroitin sulfate proteoglycans (CSPGs). Results from human postmortem studies from our group show marked decreases of PNNs in the amygdala, entorhinal cortex and prefrontal cortex of subjects with schizophrenia and bipolar disorder. Numbers of glial cells expressing the corresponding CSPGs were robustly altered, suggesting a relationship between PNN and glial abnormalities. In addition, large clusters of CSPG aggregates and glial cells, thus far morphologically and functionally poorly understood, were observed in the healthy human brain, and found to be profoundly decreased in these disorders. Finally, altered CSPG expression was observed in the olfactory epithelium of subjects with schizophrenia, suggesting widespread CSPG abnormalities, encompassing central and peripheral nervous system. Together, these findings support the hypothesis that a disruption of the interactions between PNNs, glial cell and neuronal functions may represent a key player in the pathophysiology of schizophrenia and bipolar disorder. Such disruption may contribute to some of the most well replicated aspects of the pathology of psychiatric disorders, i.e. synaptic abnormalities and neural connectivity.

S36.3
DEVELOPMENTAL ENDOPHENOTYPES IN THE PREFRONTAL CORTEX ARE REVEALED BY THE RISK FACTOR REELIN

Chavis Pascale
INSERM, 13009 Marseille, France; INMED UMR S 901, 13009 Marseille, France; Aix-Marseille Université, 13009 Marseille, France

The glycoprotein reelin is an essential building block of the brain extracellular matrix where it exhibits multiple functions both pre- and post-natally. Reelin plays an essential role in neuronal migration and positioning in the developing central nervous system1 and regulates maturation and functions of adult central synapses. Past work of our group fueled the concept that in the postnatal brain, reelin is required for the homeostasis of glutamatergic receptors that compose the majority of excitatory synapses2,3,4.

The RELN gene, which encodes for reelin, is a strong candidate in the etiology of several human psychiatric diseases including schizophrenia, autism, and mood disorders5. A number of these disorders share common features of dysfunctional prefrontal circuits and abnormal reelin expression in the brain, especially in the prefrontal cortex (PFC), thus reinforcing the link between reelin and the pathophysiology of the prefrontal cortex.

To disambiguate the pathophysiological mechanisms contributing to these disorders, we mapped the aggregate effect of the RELN risk allele on postnatal development of PFC functions by cross-sectional synaptic and behavioral analysis of reelin-haploinsufficient mice. By combining cross-sectional and multiscale phenotypical exploration with multivariate analysis of bootstrapped data sets, we identified a developmental sequence of prefrontal endophenotypes linked to the RELN risk allele. This strategy also proved useful to identify neuronal targets for pharmacotherapy. We recently reported that targeting NMDA receptors with a single in vivo injection of ketamine or Ro25-6981 induced a pharmacological restoration of reelin-haploinsufficient prefrontal phenotypes6. Ultimately, we found that this strategy advanced the disambiguation of complex traits markers underlying mechanisms of pharmacological rehabilitation.

Altogether, our data show that reelin is essential for successful structural, functional and behavioral postnatal maturation of prefrontal circuits and that Multivariate synaptic and behavioral profiling advances the disambiguation of complex traits markers underlying psychiatric disorders.

S36.4
EXTRACELLULAR MATRIX AND NEUROPLASTICITY IN HEALTH AND DISEASE

Alexander Dityatev
Molecular Neuroplasticity Group, German Center for Neurodegenerative Disorders, Magdeburg, Germany

Neural extracellular matrix (ECM) molecules derived from neurons and glial cells accumulate in the extracellular space and regulate synaptic plasticity through modulation of perisomatic GABAergic inhibition, intrinsic dendritic and axonal excitability, lateral diffusion of synaptic molecules, integrin signaling, as well as activities of L-type Ca^{2+} channels, NMDA receptors, and small GTPases (Dityatev et al., Nat Rev Neurosci, 2010). Genetic or enzymatic targeting of ECM molecules proved to bi-directionally modulate synaptic plasticity and acquisition/recall of memories, depending on experimental conditions, and to promote cognitive flexibility and extinction of fear and drug memories (Senkov et al., Progr Brain Res, 2014). Upregulation of ECM expression is associated with aging and dementia, while epileptic seizures and schizophrenia may result in attenuation of ECM. Thus, the neural ECM appeared as a key component of synaptic plasticity, learning and memory, and may be linked to cognitive dysfunctions in major

brain disorders.

S37 Room: Ballroom
GLIAL PERSPECTIVES IN BRAIN DAMAGE AND REPAIR

Chairs: Lucio Annunziato, IT and Alexei Verkhratsky, ES

S37.1
ROLE OF GLIAL CELLS IN THE RELATIONSHIP BETWEEN THYROID DYSFUNCTION AND MENTAL DISORDERS

Mami Noda

Laboratory of Pathophysiology, Graduate School of Pharmaceutical Sciences, Kyushu University, Fukuoka, Fukuoka 812-8582, Japan

Endocrine system affects the development and function of the central nervous system (CNS). Among hormones, thyroid hormones (THs) are essential not only for development of the CNS but also for neural function in adult brain. In the CNS, circulating thyroxine (T4) crosses blood-brain barrier via specific transporters and is taken up to astrocytes, becomes L-tri-iodothyronine (3, 3′, 5–triiodothyronine; T3), an active form of TH, by type 2 de-iodinase (D2). T3 is released to the brain parenchyma from astrocytes (glioendocrine system). In adult CNS, both hypo- and hyper-thyroidism, the prevalence in female being $>$ 10 times higher than that in male, may affect psychological condition and potentially increase the risk of cognitive impairment and neurodegeneration including Alzheimer's disease (AD). In mouse model of hypo- and hyper-thyroidism, sex- and age-dependent effects of THs on glial morphology were observed. Behavioral changes in hyper-thyroidism also showed sex-dependence. These results may help to understand physiological and/or pathophysiological functions of THs in the CNS. Furthermore, understanding the relationship between endocrine system and psychological condition or dementia will contribute to better diagnosis and treatment in the future.

S37.2
EMERGING ROLE OF THE SODIUM CALCIUM EXCHANGER NCX3 IN OLIGODENDROCYTES DURING CNS DEMYELINATION AND REMYELINATION

Francesca Boscia

Division of Pharmacology, Department of Neuroscience, Reproductive and Dentistry Sciences, School of Medicine, Federico II University of Naples, 80131 Napoli, Italy

A significant current drive in new Multiple Sclerosis (MS) therapeutics is to identify targets that promote remyelination by boosting endogenous oligodendrocyte precursor cells (OPC) to form new myelin before axons become irreversibly damaged. Yet, despite the ability of the adult brain to generate oligodendrocytes with myelination capacity, remyelination in MS demyelinated lesions fails or is incomplete and no neuroprotective or remyelinating therapies are still available.

Changes in intracellular $[Ca^{2+}]_i$ levels have been shown to influence the developmental processes that accompany the transition OPC into mature myelinating oligodendrocytes and are required for the initiation of myelination and remyelination processes. We explored whether calcium signals mediated by the selective sodium calcium exchanger (NCX) family members NCX1, NCX2, and NCX3, play a role in oligodendrocyte maturation. Functional studies, as well as mRNA and protein expression analyses, revealed while NCX1 was down-regulated, NCX3 was strongly up-regulated during the oligodendrocyte development. The importance of calcium signaling mediated by NCX3 during oligodendrocyte maturation was supported by the findings showing that the knocking down of the NCX3 isoform in OPC prevented the up-regulation of the myelin protein markers CNPase and MBP, whereas its overexpression induced their up-regulation.

Given the fundamental role played by NCX3 in oligodendrocytes, we explored whether NCX3 is involved in the pathophysiological responses occurring in neurons and oligodendrocytes in the spinal cord of mice exposed to myelin oligodendrocyte glycoprotein (MOG 35–55)-induced experimental autoimmune encephalomyelitis (EAE), an animal model of MS.

Western blotting and quantitative colocalization studies performed in wild-type ncx3+/+ mice at different stages of EAE showed that NCX3 protein was intensely upregulated during the chronic stage, and it was intensely coexpressed with the OPC marker NG2 and the premyelinating marker CNPase. Moreover, MOG 35–55 -immunized mice lacking ncx3 gene displayed not only a reduced diameter of axons and an intact myelin ring number but also a dramatic decrease in OPC and pre-myelinating cells in the white matter of the spinal cord when compared to ncx3+/+. Accordingly, ncx3−/− and ncx3+/− mutants developed early onset of EAE and more severe clinical symptoms. Interestingly, cytofluorimetric analysis revealed that during the peak stage of the disease, the number of immune T-cell subsets in ncx3−/− mice, was not statistically different from that measured in ncx3+/+ (Casamassa et al., 2016). In conclusion, our findings by providing evidence for the contribution of NCX3 to oligodendrocyte lineage response, demonstrated the protective role of NCX3 exchanger in EAE. Further studies are nonethe-

less needed to understand whether the selective modulation of NCX3 exchanger during demyelination may serve to enhance remyelination processes and may provide a novel therapeutic target for multiple sclerosis and, possibly, other demyelinating disorders.

S37.3
COMPLEX AND DIFFERENTIAL GLIAL RESPONSES IN ALZHEIMER'S DISEASE AND AGEING

Alexei Verkhratsky
The University of Manchester, Manchester, M13 9PT, UK

Neuroglia represented by astrocytes, oligodendrocytes, HG2 glia and microglia are homeostatic cells of the central nervous system. Neuroglial cells are also central for neuroprotection and defence of the central nervous system against exo- and endogenous insults. At the early stages of neurodegenerative diseases including Alzheimer's disease (AD) neuroglial cells become asthenic and loose some of their homeostatic, neuroprotective and defensive capabilities. Astroglial reactivity, for example, correlates with preservation of cognitive function in patients with mild cognitive impairment and prodromal AD. Here I overview the experimental data indicating glial paralysis in neurodegeneration in animal models and in human tissues and argue that loss of glial function is fundamental for defining the progression of neurodegenerative diseases.

S37.4
CNS REMYELINATION AS A NOVEL REPARATIVE APPROACH TO NEURODEGENERATIVE DISEASES – THE ROLE OF THE P2Y-LIKE RECEPTOR GPR17

Maria Pia Abbracchio
Dept. Pharm Biomol Sci, University of Milan, Italy

The P2Y-like GPR17 receptor is present on brain progenitors such as oligodendrocyte precursors (OPCs), the myelin forming cells and hippocampal double-cortin (DCX) + neuroblasts, suggesting roles in repair. In OPCs, GPR17 is needed to start differentiation but is then downregulated to allow cells' terminal maturation. Important, myelin repair correlates with neurological recovery after damage.

By also using GPR17 fluorescent reporter mice for fate mapping studies to follow the final destiny of GPR17 + OPCs, we showed that: (i) GPR17 is pathologically upregulated in trauma, stroke, Alzheimer's and multiple sclerosis; (ii) GPR17 upregulation blocks OPCs at immature stages. Thus, GPR17 antagonists counteracting aberrant GPR17 expression could help OPCs resuming maturation and foster recovery (data in progress). GPR17 antagonists could also be useful to restore cognitive functions in aging. The already marketed anti-asthmatic drug montelukast (MTK), that acts as a GPR17 antagonist induced brain rejuvenation and improved learning and memory in aged animals. Studies on neurospheres from mice lacking FOXO1, a GPR17 regulating transcription factor, and from GPR17 −/− mice showed MTK effects be due to action on GPR17/DCX + neuroblasts in hippocampal dentate gyrus, leading to increased neurogenesis.

MTK and GPR17 antagonists would thus prove useful to foster brain recovery via modulation of GPR17 on different sets of adult neural precursors. FISM2013 and ERANET Neuron RENEW IT to MPA.

S38 Room: Marie Louise 1
NEUROINFLAMMATION AND MITOCHONDRIAL DYSFUNCTION IN PARKINSON'S DISEASE

Chair: Nicola Simola, IT

S38.1
NCX1 AND NCX3 AS POTENTIAL PLAYERS IN MITOCHONDRIAL PROMOTED NEUROINFLAMMATION IN *IN VITRO* AND *IN VIVO* MODELS OF PD

Antonella Scorziello
Division of Pharmacology, Department of Neuroscience, School of Medicine, University of Naples "Federico II", Italy

The hypothesis that elevated intracellular calcium concentrations ($[Ca^{2+}]_i$) might participate to the selective degeneration of Substantia Nigra Pars Compacta (SNc) neurons has been recently taken in consideration in the pathogenesis of Parkinson's disease (PD), thus suggesting that an alteration of $[Ca^{2+}]_i$ homeostasis could be also involved in the mechanisms underlying the disease. The alterations in the levels of the Na+-Ca^{2+} exchanger isoforms (NCXs), the major cellular Ca^{2+} extruding system together with the other plasma membrane and sarco-endoplasmatic pumps in neurons and microglial cells, have been explored in mice expressing human A53T variant of α-synuclein, and correlated with the motor impairment observed in these mice during their life span. As results, Western blot studies demonstrated that in midbrain the levels of NCX2 and NCX3 isoforms were reduced during aging in A53T transgenic compared to wild type (WT) mice, whereas no differences in NCX2 and NCX3 levels have been reported in striatum. Conversely, an increase in NCX1 levels was detected in striatum during aging in A53T transgenic mice compared to WT. Interestingly, immunohistochemical studies showed that a significant increase of GFAP-

positive cells, as marker of astroglial cells, is present in striatum and in SNc, and an increase in IBA-1-positive cells, as marker of microglial cells, was observed in striatum of 10/12-months old A53T transgenic mice, as compared with WT. Moreover, 10/12-months old A53T transgenic mice exhibited a decrease in the number and in the density of TH-positive neurons in SNc, as compared with WT. These findings correlate with an age-dependent increase in the time to traverse the beam, and with a reduction in motor performance, as assessed by the open field and the pole tests, in A53T transgenic mice in comparison to WT.

In vitro experiments performed in midbrain neurons obtained from WT and A53T transgenic mice demonstrate an impairment in NCX3 levels, an increase in intracellular calcium concentration, mitochondrial calcium overload and hyperpolarization of mitochondrial membrane. Conversely, in striatal neurons no changes in NCXs levels were detected whereas cytosolic calcium concentration and mitochondrial content were lower compared to WT neurons. Interestingly, the increase in IBA-1-positive cells in striatum of 10/12-months old A53T transgenic mice correlate with an increase in NCX1, whereas TH-positive neurons and fibers in both SNc and striatum did not colocalize with NCX3, as compared with WT mice.

Collectively, these findings let to hypothesize that changes in NCXs levels and activity might play a role in mitochondrial dysfunction, microglial activation and neuronal demise observed in A53T transgenic mice thus suggesting that changes in the expression and activity of NCXs correlated to motor impairment observed in mice overexpressing the A53T variant of α-synuclein.

S38.2
MIR-34B/C ENHANCES MESENCEPHALIC DOPAMINERGIC NEURONS DIFFERENTIATION BY NEGATIVELY MODULATING THE WNT SIGNALLING

Gian Carlo Bellenchi
Institute of Genetics and Biophysics, CNR, Naples, Italy

Development and survival of mesencephalic dopaminergic neurons (mDA) is a complex and still not fully understood phenomenon. In the last years it become clear that also non-coding RNAs play important roles in promoting mDA differentiation. To better understand the role of micro-RNAs in mDA neurons development we used epiblast stem cells (epiSC), obtained from a tamoxifene-inducible Dicer KO mouse strain, differentiated towards the DA phenotype.

This approach allow investigating the contribution of miRNAs in the late phase of dopaminergic differentiation by adding tamoxifene 6–8 days after starting DA induction through SHH and FGF8 addition. By following this approach we identified miR-34b/c as a specific microRNA able to modulate WNT1 expression and facilitate proper maturation of DA neurons.

We show that miR-34b/c is able to directly bind Wnt1 3′UTR and regulate its expression. Overexpression of miR-34b/c leads to reduction of Wnt1 and Lmx1b in DA-differentiated mouse embryonic stem cells (mESc) or mouse embryonic fibroblasts (MEF). In combination with the iDA-specific transcription factors Ascl1 and Nurr1 (Caiazzo, Dell'Anno et al., 2011), miR-34b/c doubles transdifferentiation efficiency, as shown by increased TH-GFP+ neurons, providing a straightforward evidence of its implication in DA neurons development.

S38.3
INTERACTION OF MITOCHONDRIA WITH THE NUCLEUS AND ENDOPLASMIC RETICULUM IN NEURODEGENERATION

Gyorgy Szabadkai
University College London, Department of Cell and Developmental Biology, London, UK; University of Padua, Department of Biomedical Sciences, Padua, Italy

Mitochondria are key organelles determining cell fate by balancing cellular survival and death pathways. Key inputs for this function stem from functional and structural interactions of the organelle with the nucleus and endoplasmic reticulum (ER), respectively. The talk will focus on key signaling pathways involved in these interactions under cellular stress underlying neurodegenerative disease, including the unfolded protein response and DNA damage. We have shown that short-term Ca^{2+} signals transferred from the ER to the mitochondria enhance survival by promoting mitochondrial metabolism, while long term Ca^{2+} load in the mitochondria triggers cell death. At the same time ER and genotoxic stress induced nuclear gene expression reprogramming triggers mitochondrial biogenesis, which is key to maintain cellular energy balance and long term adaptation to stress.

S38.4
ANTI-INFLAMMATORY AND NEUROPROTECTIVE EFFECTS OF CANNABINOIDS IN PARKINSONIAN CONDITIONS

Gemma Navarro Brugal
Department of Biochemistry and Molecular Biology, University of Barcelona, Barcelona, Spain; CIBERNED. Centro de investigación en red en enfermedades neurodegenerativas. Instituto de Salud Carlos III. Madrid, Spain

Modulation of the levels of the endocannabinoid 2-arachidonoyl- glycerol by inhibiting monoacylglycerol

lipase alters glial phenotypes and provides neuroprotection in a mouse model of Parkinson's disease. The fatty acid amide hydrolase inhibitor, URB597, administered chronically to mice treated with 1-methyl- 4-phenyl-1,2,3,6- tetrahydropyridine and probenecid (MPTPp) over 5weeks prevented MPTPp induced motor impairment but it did not preserve the dopamine levels in the nigrostriatal pathway. The symptomatic relief of URB597 was confirmed in haloperidol-induced catalepsy assays, where its anti-cataleptic effects were both blocked by antagonists of the two cannabinoid receptors (CB1 and CB2), and abolished in animals deficient in these receptors. These results demonstrated an effect of fatty acid amide hydrolase inhibition on the motor symptoms of Parkinson's disease in two distinct experimental models that is mediated by cannabinoid receptors in both neurons and glia.

The hypothesis of direct interactions between pairs of G-protein- coupled receptors relevant for CNS function, launched by Luigi Agnati and Kjell Fuxe, has been confirmed and is now widely accepted. Natural and synthetic cannabinoids target two types of G-protein-coupled receptors (GPCRs). Cannabinoid CB1 receptors, which are enriched in the CNS and cannabinoid CB2 receptors that are more abundant in peripheral tissues. Despite the moderate expression of CB 2 receptors in brain, it has been demonstrated that CB1 and CB2 may for receptor heteromers (RHets) in the CNS. Therefore, natural or synthetic cannabinoids may act on CB1, CB2 and CB1 or CB2 containing heteromers. The research presented in this paper was undertaken to know whether these two receptors may be expressed in activated microglia. It is worth noting that current knowledge assumed that CB1 is more a neuronal than glial receptor whereas the opposite occurs for CB2. On the one hand, the expression of receptors and RHets is different in resting and activated microglia. Activation was assayed in the N9 cell line and in primary cultures of microglia using LPS and interferon gamma. On the other hand, the increase in CB1–CB2 RHets correlates with a potentiation of the effects of selective CB 2 receptors. Our results show that the composition of cannabinoid receptors and RHets in resting microglia prevent microglia activation while in conditions of microgliosis due to Parkinsonian conditions, cannabinoid agonist regulate microglial activation. The results indicate that pharmacological manipulation of cannabinoid receptors in conditions of neuroinflammation may have relevant benefits in conditions of neuroinflammation.

S39 Room: Clermont Suite
NEW INSIGHTS ON THE ENDOCANNABINOID SYSTEM: FROM THE MOLECULAR MECHANISM TO PHARMACOLOGICAL APPROACHES

Chairs: Roberto Colangeli, CA and Gordon Campbell Teskey, CA

S39.1
ENDOCANNABINOID INVOLVEMENT IN MOTOR MAP EXPRESSION AND MOTOR BEHAVIOUR

Gordon Campbell Teskey
Hotchkiss Brain Institute, University of Calgary, Canada

The CB1 receptor and endocannabinoids play a critical role in food intake, stress, pain, addiction, mood, memory and locomotor activity. The role of the CB1R and the endocannabinoids anandamide (AEA) and 2-arachidonoylglycerol (2-AG) in cortical-based movements and their representations (motor maps) is completely unknown. Determining their role is important because many emotional and cognitive based tasks use motor tests as a control for the influence of phyto-, synthetic-, and endocannabinoids. Moreover endocannabinoids have been shown to affect spinal mediated locomotion and a more complete understanding of their role in movement is needed because of increasing usage of phytocannabinoids and synthetic cannabinoid drugs.

We used short duration intracortical microstimulation (SD-ICMS) to derive movement representations of the forelimb and the single pellet reaching task to examine forelimb behaviour.

Our results indicate that both AEA and 2-AG contribute to forelimb motor map expression by acting through CB1 receptors, exert a dampening effect on forelimb motor map expression but have little effect on forelimb behaviour.

S39.2
REGULATION OF SYNAPTIC EFFICACY BY ANANDAMIDE TRANSPORT THROUGH PANNEXIN-1

Roger Thompson
Hotchkiss Brain Institute, University of Calgary, Calgary, Alberta, Canada

Pannexin-1 (Panx1) are ion and metabolite permeable channels with a broad distribution in the brain. In the hippocampus, they are best characterized at the CA3-CA1 synapse, where they contribute to excitotoxicity. The physiological functions of Panx1 are not well characterized. Here we show that selective block or knockout of Panx1 in CA1 neurons (postsynaptic) augments stimulation-induced glutamate release from CA3 neurons (presynaptic). This manifested as a short ($\sim$ 10 s) increase in the frequency of mini excitatory postsynaptic potentials (mEPSP) without a concomitant change in the amplitude. Increased frequency of mEPSPs sug-

gests a presynaptic effect of altering postsynaptic Panx1 channels. Augmented presynaptic glutamate release required the activity of transient receptor potential channels, TRPV1, because it was absent in TRPV1−/− mice and in the presence of capsazepine. Given that TRPV1 is activated by the endocannabinoid, anandamide (AEA), we hypothesized that Panx1 was regulating the synaptic levels of AEA. Surprisingly, Panx1 was found to be permeable to AEA as evident by direct block of dye flux through Panx1 by AEA. Furthermore, direct postsynaptic loading with AEA spontaneously increased mEPSP frequency in a Panx1 and TRPV1 dependent manner. We conclude that Panx1 functions as an important mechanism for regulating synaptic levels of AEA and that when blocked, Panx1 can enhance the short-term excitability of CA3-CA1 synapses.

S39.3
EPILEPSY AND COMORBIDITIES: INVOLVEMENT OF THE ENDOCANNABINOID SYSTEM

Roberto Colangeli
Hotchkiss Brain Institute, Dept. of Cell Biology and Anatomy, University of Calgary, Calgary, AB, Canada

Comorbid psychiatric disorders are common in patients with epilepsy. These comorbidities include depression, anxiety, psychoses, and cognitive dysfunction. It has been extensively documented that temporal lobe epilepsy, the most prevalent form of adult epilepsy in humans, is often associated with memory deficits and negative emotional disturbances. The endocannabinoid (eCB) system is a neuromodulatory system in the brain which finely regulates neuronal excitability and synaptic plasticity. In both humans and rodents, cannabinoid type 1 receptor and all the machinery responsible for the synthesis and degradation of eCBs are highly expressed in the hippocampus and amygdala. Data presented here show how the eCB system is involved in hippocampal and amygdalar excitability and synaptic plasticity following seizures. Furthermore, our laboratory recently characterised a long-lasting period of hypoxia which occurs after the termination of a seizure and may be causally related to the behavioural comorbities. Here, the potential involvement of eCBs in the long-lasting postictal hypoxia is presented. Overall these data suggest that pharmacological eCB modulations might be a suitable approach for the treatment of epilepsy dampening the comorbid disorders associated with epilepsy.

S40 Room: Reading Room
TARGETING NEUROSTEROIDOGENESIS FOR THE TREATMENT OF NEURODEGENERATIVE AND NEUROPSYCHIATRIC DISEASES

Chair: Roberto Frau, IT

S40.1
5α-REDUCTASE INHIBITION AS A POSSIBLE TREATMENT FOR DYSKINESIAS IN PD

Silvia Fanni
Dept. of Biomedical Sciences, University of Cagliari, S.S. 554 Bivio Sestu, Monserrato, 09042, Italy

Long-term administration of l-3,4- dihydroxyphenylalanine (L-DOPA), the mainstay therapy for Parkinson's disease (PD) patients, is accompanied by the development of dyskinesia, a disabling motor complication that dramatically affects patients' quality of life. L-DOPA induced dyskinesias (LID) have consistently been related to abnormal dopaminergic transmission, with dysfunctions in down-stream signalling of the striatal D 1 receptors as the most characterizing feature.

Inhibition of 5α-reductase (5AR), the rate-limiting enzyme for neurosteroids synthesis, has been shown to elicit marked anti-dopaminergic effects. Indeed, we previously found that the 5AR inhibitor finasteride (FIN) is able to normalize several behavioral alterations induced by the administration of dopaminergic agonists, through a post-synaptic modulation of the D1 receptor in the striatum. In light with these evidence, we hypothesized that Inhibition of 5AR may also counteract dyskinesia in 6-OHDA- lesioned rats by normalizing the striatal D 1 receptor signalling cascade.

In fact, chronic treatment with different doses of finasteride (30–100 mg/kg, IP) significantly reduced development of dyskinesia induced by both L-DOPA and the selective D 1 agonist (R)-(+)-SKF-38393. This effect was accompanied by a marked reduction of the striatal D 1 receptor-related Shp-2/Erk1/2 signalling pathway. In addition, chronic finasteride administration reduced the severity of established LID.

To our knowledge, this is the first study that highlights a possible role of 5AR and its related neurosteroids in the pathophysiology of L-DOPA induced dyskinesia, and suggests FIN as a promising therapeutic agent for the treatment of dyskinesia. The clinical relevance of these findings are further supported by the fact that FIN has recently been proven effective in pathological conditions related to abnormal striatal dopamine transmission, such as Tourette Syndrome and impulse control disorders.

S40.2
THE 5α-REDUCTASE INHIBITOR DUTASTERIDE BUT NOT FINASTERIDE PROTECTS DOPAMINE NEURONS IN THE MPTP MOUSE

MODEL OF PD

Therese Di Paolo
Neuroscience Research Unit, Centre Hospitalier Universitaire de Québec, CHUL, Quebec City, Canada; Faculty of Pharmacy, Laval University, Quebec City, Canada

Parkinson's disease (PD) is the most common neurodegenerative movement disorder and is likely to increase due to the aging population. There is no cure for PD and no disease-modifying drug available. Current treatments available are only symptomatic. A higher incidence of this disease is observed in men suggesting a possible neuroprotective effect of female sex steroids. Indeed, an abundant literature has documented the neuroprotective effects of estrogens in animal models of PD. We have explored the neuroprotective activity of drugs affecting steroid synthesis. Dutasteride and finasteride are inhibitors of the enzyme 5α-reductase used in humans to treat various endocrine conditions. In the mouse model of PD lesioned with 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) we found that dutasteride (5.0 and 12.5 mg/kg) but not finasteride (5.0 and 12.5 mg/kg) exhibits neuroprotectionve activity in the brain of dopamine (DA) neurotransmission and various associated markers. The mechanisms of action of the neuroprotective effects of dutasteride were further investigated in intact and MPTP-lesioned mice. C57Bl6 male mice were treated with dutasteride (5 or 12.5 mg/kg) once daily for 10 days. Mice received 4 injections of MPTP (5.5 mg/kg) or saline on the 5th day. Analysis of the motor behavior of these mice showed that treatment with MPTP or MPTP + dutasteride did not affect their motor performance as expected using a low dose of MPTP to induce a moderate lesion modeling early stages of the disease. In these mice dutasteride prevented the depletion of striatal DA as well as its metabolite DOPAC measured by HPLC while serotonin concentrations remained unchanged. DA and its metabolite levels were unchanged by dutasteride treatment in intact mice. Autoradiography of striatal dopamine transporter (DAT), vesicular monoamine transporter 2 and D2 DA receptor specific binding showed that treatment with dutasteride decreased the effect of the toxin while striatal D1 receptor specific binding remained unchanged by the MPTP lesion and the dutasteride treatment. Dutasteride enhanced striatal DAT specific binding and the glycosylated form of DAT in intact mice as measured by Western blot. DAT mRNA measured by in situ hybridization in the substantia nigra remained unchanged by the MPTP lesion and the dutasteride treatment at 5 mg/kg while it decreased with dutasteride treatment at 12.5 mg/kg in both intact and lesioned mice. MPTP-lesioned mice had levels of plasma and brain dihydrotestosterone (DHT) significantly lower than control mice. Dutasteride treatment elevated plasma and brain concentrations of testosterone compared to control and MPTP mice and decreased DHT levels. Striatal glial fibrillary acidic protein (GFAP) levels were markedly elevated in MPTP-lesioned mice compared to control mice and dutasteride reduced GFAP levels in MPTP-lesioned mice suggesting an anti-inflammatory activity of dutasteride. PD also presents with non-motor symptoms such as gastrointestinal dysfunctions that are very common, significantly reducing the quality of life of patients and starting before motor symptoms appear. In the present mice experiments we also collected the guts to study the enteric nervous system that we previously reported to be altered in MPTP-lesioned mice. Dutasteride prevented the MPTP-induced loss of Tyrosine Hydroxylase-immunoreactive neurons in the myenteric plexus. Moreover, the increased macrophage density following the MPTP lesion was prevented by dutasteride, highlighting its immunomodulatory properties. Dutasteride also prevented the 1-methyl-4- phenylpyridinium-induced production of nitric oxide and reactive oxygen species in human monocytic cells in vitro. In another experiment in MPTP-lesioned mice treatment with dutasteride, started after the MPTP lesion for 5 days, left unchanged striatal DA and metabolites levels. Taken together these results suggest that dutasteride exhibited neuroprotective effects on DA neurons and anti-inflammatory properties both in the brain and the gut. These results propose dutasteride as promising therapeutic drug for neuroprotection in early stages of PD.

S40.3
SLEEP DEPRIVATION INDUCES MANIC-LIKE BEHAVIOURS THROUGH ALTERATIONS IN NEUROSTEROIDOGENESIS

Roberto Frau
Dept. of Biomedical Sciences, Division of Neuroscience and Clinical Pharmacology, University of Cagliari, Italy; Tourette Syndrome Center, University of Cagliari, Italy; Sleep Medicine Center, University of Cagliari, Italy

Acute sleep deprivation (SD) can trigger or exacerbate psychosis- and mania-related symptoms; the neurobiological basis of these complications, however, remains elusive. Given the extensive involvement of neuroactive steroids in psychopathology, we hypothesized that the behavioural complications of SD may be contributed by 5α-reductase (5αR), the rate-limiting enzyme in the conversion of progesterone into the neurosteroid allopregnanolone. We first tested whether rats exposed to SD may exhibit brain-regional alterations in 5αR isoenzymes and neuroactive steroid levels; then, we assessed

whether the behavioral and neuroendocrine alterations induced by SD may be differentially modulated by the administration of the 5αR inhibitor finasteride, as well as progesterone and allopregnanolone. SD selectively enhanced 5αR expression and activity, as well as AP levels, in the prefrontal cortex; furthermore, finasteride (25–100 mg/kg, IP) ameliorated PPI deficits, hyperactivity, and risk-taking behaviors, in a fashion akin to the antipsychotic haloperidol and the mood stabilizer lithium carbonate. Finally, PPI deficits were exacerbated by allopregnanolone (10 mg/kg, IP) and attenuated by progesterone (30 mg/kg, IP) in SD-subjected, but not control rats. Collectively, these results provide the first-ever evidence that 5αR mediates a number of psychosis- and mania-like complications of SD through imbalances in cortical levels of neuroactive steroids.

S40.4
EFFECTS OF FINASTERIDE IN THE NERVOUS SYSTEM: FOCUS ON NEUROACTIVE STEROIDS

Silvia Giatti
Department of Pharmacological and Biomolecular Sciences, Università degli Studi di Milano, Milan, Italy

Neuroactive steroids are a broad class of molecules able to affect nervous functions. They can be produced by peripheral steroidogenic glands as well as in the nervous system (NS), where they act as modulators in physiological conditions. Their central role has been confirmed in pathological situations, since their levels are altered in neurodegenerative diseases or after trauma in NS. Hence, any perturbation in neurosteroidogenic pathway could result in NS dysfunctions. To evaluate this hypothesis, we studied the administration of finasteride, an inhibitor of the enzyme 5α-reductase (5α-R), which catalyzes the conversion of progesterone and testosterone in dihydroprogesterone (DHP) and dihydrotestosterone (DHT), respectively. This conversion is crucial for steroid mechanism of action. In particular, DHP is the precursor of allopregnanolone, a molecule known to be involved in mood regulation. On the other hand, DHT is the active metabolite of testosterone, since DHT interacts with more affinity with the androgen receptor. Considering its crucial role in neuroactive steroid action, finasteride has been mainly used in experimental models as pharmacological tool to discriminate the effects of progesterone or testosterone with respect to their metabolites. However, the consequences of finasteride administration per se, in physiological conditions, received poor attention.

It is important to recall that finasteride is also commercially available to treat benign prostatic hyperplasia and androgenetic alopecia (male pattern hair loss) in men. It has been reported that finasteride treatment could produce some side effects that generally disappear after stopping the drug. However, in a small subset of men taking finasteride for alopecia, some side effects persist after withdrawal. This condition is called post-finasteride syndrome (PFS) and accounts for sexual side effects and mood disorders. Studies conducted in plasma and liquor from PFS patients revealed alterations in neuroactive steroid levels.

Recently, we studied the effect of finasteride treatment in NS of male rats. In particular, the effects of 3 mg/kg injections of finasteride for 20 day have been evaluated after subchronic treatment and after one month of withdrawal. Interestingly, levels of neuroactive steroids and the expression of steroid receptors were altered in treated animals, and more intriguing, they are differentially modified depending on the time point and on the cerebral structure considered. Overall, our data reported alterations in the NS after finasteride treatment that persist time after drug discontinuations. These evidences might help to understand the mechanisms underpinned in the persistent side effects observed in PFS patients.

S40.5
UNRAVELLING THE MOLECULAR MECHANISM GOVERNING THE ANTIPSYCHOTIC-LIKE EFFECTS OF FINASTERIDE

Simona Scheggi
Dept. of Pharmacology and Toxicology, College of Pharmacy, University of Utah, Salt Lake City (UT); Department of Molecular and Developmental Medicine, University of Siena, Siena, Italy

The enzyme 5α reductase 2 (5αR2) catalyzes the conversion of progesterone and testosterone into neuroactive metabolites that are known to be potent allosteric modulators of the GABA A receptor and are involved in depression, memory, social and sexual behavior. 5αR2 is present in key brain areas that regulate emotional behavioral responses, raising the possibility that 5αR2 may be implicated in the regulation of emotion and mood. To test for this possibility, we studied the behavioral phenotypes of 5αR2 knockout (KO) and heterozygous (HZ) mice, as compared with their wild-type (WT) littermates. Our results evidenced that 5αR2 KO male mice displayed reduced preference for natural rewards (saccharin and sex), as well as reduced proclivity to novelty, as signified by their reduced exploration of novel objects and the open arms of an elevated plus maze. Finally, 5αR2 KO male mice displayed a reduced proclivity to reach dominance status. No significant differences were found in sensory, motor and mnemonic functions. To identify the biological substrates responsible for the motivational impairments in 5αR2 KO mice, we characterized the levels of expression of dopamine receptors

in their mesocortical systems. Our results showed that 5αR2 KO mice exhibited a significant upregulation of D1 dopamine receptors in the striatum, but not in other brain regions, in comparison with WT controls. Taken together, these results provide the first-ever demonstration that 5αR2 is directly involved in the regulation of incentive behaviors, possibly through dysregulations of dopamine receptors.

S41 Room: Marie Louise 2
APPLICATION OF T-PATTERN DETECTION AND ANALYSIS (TPA) FOR THE DISCOVERY OF HIDDEN FEATURES OF HUMAN AND ANIMAL BEHAVIOUR

Chairs: Maurizio Casarrubea, IT and Magnus S. Magnusson, IS

S41.1
INTEGRATING CATEGORICAL AND CONTINUOUS DATA TO ANALYSE THE EFFECTS OF A VARIED EXERCISE WORKOUT PROGRAM IN ADULT WOMEN

Marta Castañer
INEFC, University of Lleida. Spain

In order to understand and improve the scenarios to be managed by teachers it is important to identify the essential aspects of communication, such as gestures, voice quality and the use of teaching time and space, which are associated with the teaching discourse. We focus on how to analyse the paraverbal communicative fluency of teaching style. Essential paraverbal criteria related to kinesics and proxemics were studied in lecturers offering courses. Some lessons were analysed using the Observational Systems of Paraverval Communication SOCIN and SOPROX, both observational instruments that enables a broad analysis of kinesics and proxemics. The Theme software was used to detect temporal patterns (T-patterns) in the observational data. The results reveal the power of the teachers'; illustrative and regulatory kinesics. The regulatory function makes use of clearly-defined kinesic gestures such as emblems and kinetographs, whereas the illustrative function is accompanied by largely undefined kinesic gestures.

S41.2
FROM SEQUENTIAL ANALYSIS TO SYMMETRY AND SELF-SIMILARITY: DETECTING RECURRENT HIERARCHICAL STRUCTURED TEMPORAL CLUSTERS IN BEHAVIOUR

Magnus Magnusson
Human Behavior Laboratory, University of Iceland, Reykjavik, Iceland

Behavioral analysis has for long been characterized by the use of standard statistical methods developed in other contexts, that is, for quantitative analysis of particular other phenomena or any quantifiable phenomena. In behavioral research the counting of behavioral events and states and the measuring of their frequencies and durations has long dominated. Multivariate statistics have also been used to discover relations between behaviors and/or subjects. Hierarchical Cluster Analyses represent such relations in terms of hierarchies of clusters of clusters, but like with Factor Analysis, the clusters or factors do not describe patterns that recur in time like, for example, repeated words or routines as patterns of simpler behaviors. Standard statistical methods such as sequential analysis are more rarely used partly due to over-simplifying assumptions often preventing the detection of even abundantly recurring patterns. The T-pattern model with its extensions, called the T-system, and corresponding detection algorithms and software, Theme (for Windows), were developed to make such pattern detection feasible and easily available even if computationally intensive. Searching for complex repeated patterns holds the promise of thereby detecting effects of independent variables missed by other methods as research increasingly indicates notably in neuroscience. Pattern Detection and Analysis (TPA) with Theme (see hbl.hi.is & www.patternvision.com) allows the analysis of quite voluminous data, but due to the particular and intensive use of temporal information also of tiny data (even just a few events). The T-pattern model, the T-system and the corresponding algorithms are described together with illustrative applications and results.

S41.3
APPLICATION OF T-PATTERN DETECTION AND ANALYSIS (TPA) FOR THE DISCOVERY OF HIDDEN FEATURES OF HUMAN AND ANIMAL BEHAVIOUR

M. Teresa Anguera
Faculty of Psychology, Institute of Neurosciences, University of Barcelona, Spain

The growing body of scientific literature in human behavior shows the complementary nature of qualitative and quantitative data. The aim of this work is to highlight the enormous potential that indirect observational methodology offers in this respect, and how both approaches produce qualitative data that can be quantified. Indirect observational methodology implies that, even several decades ago, researchers gathered and used textual information, such as life stories, self-reports, per-

sonal accounts, narrative registers or any other type of written document.

Before transforming qualitative inputs from human behavior into quantitative data, it is first necessary to decide how to organize the heterogeneous information available. This process can be extremely complex as it is possible to bring together data from very different sources, and perhaps, different points in time. The first step is to correctly record and code the data. As started by Bradley, Curry, and Devers (2007:1761), "coding provides the analyst with a formal system to organize the data, uncovering and documenting additional links within and between concepts and experiences described in the data".

Human communication flows offer enormous research potential thanks to their multiple dimensions or levels of response and their extraordinary dynamic nature. Their study, however, poses methodological challenges, beginning with the establishment of dimensions or response levels (known as variables in THEME) and the criteria used to segment episodes into behavioral units, which give rise to event types and their arrangement into separate blocks.

It is relevant to establish text segmentation criteria to produce a series of textual units. The equivalent of these units in direct observation is observation units. Krippendorf (2004) suggested several criteria, either alone or combined, to systematically segment texts. However, we consider this to be insufficient. This initial stage is crucial as the categories created will directly determine the information that will emerge from the set of data. Where possible, test runs or pilot studies should be performed first. The ad hoc instrument of observation that it is idoneous is a combined field format and category system. The field format can be built from observation or textual units that emerge from the segmentation of a texts.

The core is the transformation of a text in a matrix of codes that can be analyzed quantitatively. The columns correspond to the dimensions established, the rows to the units in which they were segmented, and each row shows co-occurrence coding. The time units are conventional. The complexity of human behavior results in a record of episodes adapted to the syntactic rules of THEME, so that the invisible structure to which it adapts and by which it is regulated can be extracted and studied quantitatively. The invisible nature of T-patterns increases the potential for discovery, as researchers are interested in extracting the internal structure that unveils the key to the target behaviors.

S41.4
T-PATTERN ANALYSIS IN THE STUDY OF RODENT BEHAVIOUR. METHODOLOGICAL AND EXPERIMENTAL HIGHLIGHTS

Maurizio Casarrubea
Laboratory of Behavioral Physiology, Dept. of Bio.Ne.C., School of Medicine, University of Palermo, Italy

The lack of information concerning the temporal characteristics is a common aspect of the largest amount of experimental approaches to the study of behavior. To fill this gap the T-pattern analysis can be used. Such a technique has been developed to determine whether two or more behavioural events occur sequentially and with statistically significant time intervals. The utilization of such an approach has produced, in recent years, several interesting results, both in human and animal research. In our laboratories we have successfully applied T-pattern analysis to study rat behavior in various experimental assays such as the open field, the hole board, the elevated plus maze and the hot-plate. By using Theme software and related procedures of T-pattern detection and analysis, we have observed that numerous events, characterizing rodent behavior in each experimental model, occurred sequentially and with significant constraints on the interval lengths separating them. In this presentation, for each test, various key results will be presented and discussed. In addition, by highlighting methodological details of our behavioral analyses, useful information concerning the application of T-pattern analysis in the study of behavior will be provided.

S41.5
EXPANDING THE STUDY OF INTERNET GAMBLING BEHAVIOUR: PATTERNS AND TRENDS WITHIN THE ICELANDIC LOTTERY AND SPORTS BETTING PLATFORM

Jonsson K. Gudberg
Human Behavior Laboratory, University of Iceland, Iceland

As rates of Internet gambling participation increase worldwide, so too does the need to understand how people engage in this form of gambling. This study represents the first examination of actual Internet gambling records within Iceland, a Nordic country with an active Internet lottery market that imposes strict regulations on gambling operator licenses. We summarized electronic betting records of a cohort of subscribers to the Internet betting service provider Íslensk Getspá. In addition, we search for temporal patterns in individual gambling records. We observed that the typical subscriber bet approximately 3 days per month and made fewer than two bets per gambling day, each worth approximately the equivalent of $4 US. Subscribers lost the bulk (96 %) of the amount they wagered, for a total loss of approximately $40 across the 2-year window of

observation. Although these observations do not support the view of Internet gambling as an activity that is inherently risky for the typical subscriber, we did observe discontinuity across the distributions of gambling behavior, with the top 1% of subscribers making more than three bets per day. Certain betting pattern were exclusively detected with frequent gamblers and different pattern types between gender and age groups.

S42 Room: Carlson Suite
EXPRESSION AND INHIBITION OF CONDITIONED FEAR

Chair: Markus Fendt, DE

S42.1
THE ROLE OF ACTIN CYTOSKELETON AND ITS REGULATORY PROTEINS IN CONSTRAINING FEAR MEMORY FORMATION IN AMYGDALA

Raphael Lamprecht
Sagol Department of Neurobiology, University of Haifa, Haifa, Israel

Experiencing a traumatic event may lead to the development of fear-related disorders in some individuals but not in others. It is therefore important to understand the cellular and molecular mechanisms that generate and constrain the formation of long-term fear memory. While there are a number of experimental tools for studying fear and anxiety, one of the simplest and most straightforward is fear conditioning. We have examined the role of actin cytoskeleton in fear conditioning memory formation. The actin cytoskeleton which is regulated by synaptic transmission controls key neuronal functions such as synaptic transmission and morphogenesis. Actin cytoskeleton may therefore mediate between fear learning and long-term cellular alterations mandatory for fear memory formation and be involved in constraining the generation of fear memory. We have found that the activities of several actin-regulatory proteins constrain the formation of fear memory in lateral amygdala (LA), known to mediate fear conditioning. One of these actin-regulatory protein is the myosin light chain kinase (MLCK), a calcium/calmodulin-dependent protein kinase that phosphorylates the myosin regulatory light chain (RLC), leading to contraction of the actomyosin filaments. Inhibition of MLCK in LA before fear conditioning, but not immediately afterward, enhanced both short-term memory and long-term memory. Notably, the MLCK inhibitor had no effect on memory retrieval. Anatomical studies show that MLCK is present in cells throughout LA and is localized in dendritic shafts and spines that are postsynaptic to the projections from the auditory thalamus to LA, a pathway implicated in fear learning. Inhibition of MLCK enhanced long-term potentiation, a physiological model of memory, in the auditory thalamic pathway to LA. We also found that Rac GTPase and its downstream effector p21-activated kinase (PAK), known to affect actin cytoskeleton polymerization and structure, constrain fear memory formation in amygdala. To assess possible roles of Rac GTPase in fear memory we used a photoactivatable form of Rac1 (PA-Rac1) that can interact and activate its effector proteins when stimulated by light. Activation of PA-Rac1 in LA by light led to phosphorylation of PAK. PA-Rac1 activation in LA during fear conditioning learning trials impaired long-term but not short-term fear memory formation. Inhibition of PAK in LA by microinjection of the PAK inhibitor IPA-3 30 minutes before fear conditioning enhanced long-term but not short-term fear memory formation. Cumulatively, our results indicate that the actin cytoskeletal regulatory proteins MLCK, Rac1 and PAK suppress fear conditioning memory, suggesting that these proteins are involved in blunting the acquisition of irrelevant fears. Impairment of this multi protein-based mechanism could contribute to pathological fear learning.

S42.2
IMPACT OF BDNF/TRKB-SIGNALING ON FEAR EXPRESSION AND FEAR INHIBITION

Thomas Endres
Institute of Physiology, Medical Faculty, Otto-von-Guericke University Magdeburg, Magdeburg, Germany

The neurotrophin BDNF (brain-derived neurotrophic factor) is an important mediator of synaptic plasticity and is crucially involved in learning and memory processes. Several recent studies demonstrated that BDNF is also important for fear learning as well as for the extinction of fear. However, the mechanisms how BDNF contributes to these learning mechanisms remain still widely unknown. Therefore, we used a combined approach of behavioral physiology with in vitro and *ex vivo* electrophysiology to further unravel the role of BDNF in these learning tasks. By local pharmacological interference with BDNF-TrkB signaling in the basolateral amygdala (BLA) we could identify distinct time windows for the acquisition and consolidation of cued fear memories in mice. Furthermore, we could recently demonstrate that heterozygous BDNF knock-out (BDNF +/−) mice exhibit an age-dependent learning deficit when they are three months old or beyond. Interestingly, BDNF-dependent long-term potentiation (LTP) at cortical input synapses to the lateral amygdala as well as at subsequent glutamatergic intra-amygdala synapses was not

affected in these animals. A detailed analysis of the early fear memory revealed that short-term fear memory was present in BDNF +/− mice up to 6 h after training, but the precision of the memory gradually declined, suggesting an intact acquisition of fear memories. By *ex vivo* LTP recordings of previously fear conditioned mice, we could demonstrate that fear learning leads to long-lasting changes at cortical input synapses to the lateral amygdala, as indicated by occlusion of LTP. In contrast, these long-lasting changes were absent in BDNF +/− mice at these synapses, mirroring the observed deficit in fear memory consolidation. Thus, our data support the important role of cortical input synapses to the lateral amygdala for fear learning and suggest that intact LTP at these synapses in BDNF +/− mice could mediate acquisition but not consolidation of cued fear memories. Besides fear learning we are interested in the involvement of BDNF/TrkB-signaling in the extinction of conditioned fear. Therefore, we started to analyze the time course of BDNF protein expression in different regions that have been identified to be involved in fear extinction processes, i.e., BLA, hippocampus and medial prefrontal cortex (mPFC). Here we observed distinct peaks of BDNF protein expression in the amygdala and mPFC but not in the hippocampus at different time points after fear extinction training (0–120 min). Interestingly, the time courses of BDNF expression differed between mPFC and BLA, suggesting a sequential contribution of BDNF/TrkB-signaling in these brain areas during the consolidation of fear extinction memories. Currently we are performing pharmacological experiments to locally interfere with BDNF/TrkB signaling in these brain areas in order to pinpoint the required dynamics of BDNF/TrkB signaling that are mandatory for the successful consolidation of fear extinction memories. In conclusion, our results support the important role of BDNF/TrkB-signaling for fear conditioning and fear extinction learning and reveal more detailed insights into the location and dynamics of this signaling pathway in these learning processes.

S42.3
LEARNING CATEGORIZATION OF FEAR AND SAFETY LEARNING THROUGH EXPERIMENTAL AND COMPUTATIONAL EYES

Radwa Khalil
Center for Molecular and Behavioral Neuroscience, Rutgers University, Newark, USA; Behavioral and Neural Sciences Graduate Program, Rutgers University, Newark, USA

Exaggerated fear is one of the lead symptoms in anxiety disorders. Therefore, it is crucial to gain better knowledge about the mechanisms underlying fear. Whereas previous research was more focused on the mechanisms of fear learning and fear expression, recent research more and more focus on the question of how fear is inhibited. The most known example of fear inhibition is extinction learning however there are additional phenomenona like safety or relief learning. In safety learning, stimuli are associated with the absence of an aversive event, whereas in relief learning, stimuli are associated with the offset of an aversive event. Notably, safety learning is impaired in patients with anxiety disorders. All these learning processes are not only observed in humans but also in other mammals such as laboratory rodents. Notably, fear and relief learning, but not safety learning, was also investigated in fruit flies (Drosophila melanogaster).

Recent studies demonstrate that relief learning (but not safety learning) is mediated by the brain system mediating reward. This system is connected with the brain system mediating fear and punishment. This led to the hypothesis that a competition between these two systems could be involved in fear and relief learning, respectively.

Here, we provide a computational neural network model that simulates interactions among the mammals' or flies' brain systems mediating fear/punishment and relief (mammals: amygdala and nucleus accumbens; flies: Kenyon cells). Our model will simulate fear and relief learning through competition among these neural systems. This model could be used as predictive tool to guide further behavioral and/or mechanistic studies in animals and humans.

S42.4
AMYGDALA CIRCUITS AND MECHANISMS THAT CONTROL OF ACQUIRED FEAR AND ITS EXTINCTION

Ingrid Ehrlich
Hertie Institute for Clinical Brain Research and Centre for Integrative Neuroscience, University of Tuebingen, Germany

My lab uses Pavlovian cued fear conditioning and its extinction to investigate the substrates and mechanisms underlying the expression of learned fear and safety. A key region for emotional stimulus-associations and storage of fear and extinction memories is the amygdala. The amygdala directly receives sensory inputs and is part of a larger interconnected network with hippocampus and medial prefrontal cortex, which has been implicated in state- and context-dependent control of fear. Increasing evidence suggests that parallel processes in these circuits and inhibitory elements control fear and extinction memory.

We employ a combination of behavioral, and *ex vivo* electrophysiological, anatomical and optogenetic ap-

proaches in mice to delineate properties of and plasticity in fear and extinction circuits. My talk will highlight several aspects of our recent work. On the network side, I will discuss data on the functional architecture of prefrontal- and hippocampal-basolateral amygdala circuits, and the role of a specific set of local inhibitory synapses in the basolateral amygdala that participate in extinction. From a systems perspective, I will show that sleep supports the consolidation of fear extinction memory and discuss preliminary data on the associated global activity patterns during sleep. Lastly, I will describe novel connectivity of amygdala intercalated cells, a specific set of GABAergic neurons surrounding the BLA which were thought to play a key role in extinction. Our data suggest that these cells may also participate in fear learning and its expression, as they provide learning-modulated sensory feed-forward and feedback inhibition to basolateral amygdala. This puts them in a unique position to gate fear expression or suppression.

S42.5
MIRNA AND LEARNED SAFETY

Marianne Ronovsky
Department of Neurophysiology and Neuropharmacology, Center for Physiology and Pharmacology, Medical University of Vienna, Austria

As fear can be generated and increased by learning processes, likewise also learned inhibition mechanisms of fear do exist. Conditioned inhibition of fear is a fear inhibitory mechanism, which involves learning of safety signals and is therefore also referred to as learned safety. Learned safety signals are effective beyond the regulation of fear responses and identification of episodes of security as they also relate to positive affective conditions, eliciting reward-related approach and a reduction of depression-like behavior in mice. While some selected insights into the neural underpininngs have been obtained and evidence for its translational potential exist, the molecular mechanisms of learned safety remain incompletely understood. We here examined the role of microRNAs (miRNAs), small noncoding RNAs, which modulate gene expression at the posttranscriptional level in learned safety. Specifically, we investigated the contribution of selected miRNA species (of the miR-212/132 family) on the behavioral expression of learned safety and on synaptic activity in the basolateral amygdala, a brain region central to the neural circuitry of learned safety. *In-silico*, *in-vitro* and *ex-vivo* approaches were then combined to identify possible relevant target genes of miRNA-132 mediating the neural effects of learned safety. Learned safety is a relatively unexplored tool for neuropsychiatric research along the newly established Research Domain Criteria system where it can be applied as paradigm within the sub-construct "reward prediction" of the "positive valence" system. The present results integrate a wide range of analytical levels, serve to elucidate some of the relevant molecular processes of this behavioral state and contribute to the completion of the matrix which can be applied to characterize the "learned safety-domain" from all different angles amenable to neuroscientific research.

S43 Room: Ballroom
TRANSVERSAL SYMPOSIUM: INITIATIVES FOR EURO-MEDITERRANEAN COLLABORATIONS - ROUND TABLE

Chairs: Demian Battaglia, FR and Mohammad Herzallah, USA

Mohammad Herzallah, Ahmed El Hady, David Hansel, Marc Landry and Driss Boussaoud

S44 Room: Carlson Suite
INSIGHTS ON THALAMIC FUNCTION: A VIEW FROM NEUROPSYCHIATRIC DISORDERS

Chair: Mohamed Bennis, MA

S44.1
EARLY POSTNATAL ALTERATION OF THE THALAMOCORTICAL PATHWAY LEADS TO SOME SCHIZOPHRENIA-LIKE SYMPTOMS

Mohamed Bennis
Lab Pharmacology, Neurobiology & Behavior, Cadi Ayyad University, Marrakech, Morocco

Schizophrenia is a complex psychiatric condition affecting 1% of the world's population. It is characterized by profound disturbances of mental functions and subtle brain abnormalities. A number of developmental models were subsequently generated that allowed testing of hypotheses about the origin of the disease, mimicked a wider array of clinical and neurobiological features of schizophrenia, and opened new avenues for developing novel treatment strategies. Altered activity in the prefrontal cortex (PFC) has been associated with these cognitive symptoms. However, the cognitive symptoms of schizophrenia, typically linked to prefrontal cortex (PFC) dysfunction remain essentially resistant to treatment. Thus, it remains critical to understand the underlying neural mechanisms of these symptoms in order to target effective treatments, because cognitive symptoms are highly predictive for the long-term prognosis of the disease.

Recently, the mediodorsal thalamus (MD) has become a focus of attention in the study of cognitive symptoms and schizophrenia, mainly due to its dense excitatory re-

ciprocal connections with the PFC. Neuroimaging and postmortem anatomy in schizophrenic patients indicates that MD is shrunken with neuronal loss, or has metabolic changes. Combining a plethora of behavioral paradigms, we are addressing the longer-term cognitive and behavioral effects of early developmental insult to MD in adult rats. An important platform of cognitive and behavioral tasks assessed the generality of social interactions and anxiety, as well as learning, memory, strategy shifting and latent inhibition.

Very interestingly, we have shown that early MD lesions sustained at day P4, led in early adulthood to the emergence of a complex syndrome encompassing positive, negative and cognitive-like symptom components resembling to that observed in schizophrenia.

S44.2
THE IMPLICATION OF EARLY THALAMOCORTICAL CROSS-TALK IN THE DYNAMIC BALANCE OF EXCITATION AND INHIBITION IN RAT BRAIN

Zakaria Ouhaz

Lab Pharmacology, Neurobiology & Behavior, Cadi Ayyad University, Marrakech, Morocco

It is believed that neuronal networks *in vivo* function in a "balanced" regime, where excitatory and inhibitory (E/I) neuron interactions maintain tightly correlated levels of activity. It is not only considered to be a functional cornerstone in the cerebral cortex, but also has been hypothesized to play a major role in areas other than cortex.

The establishment of E/I balance in adult cortical neurons may result from developmental and experience-dependent co-regulation of developing glutamatergic and GABAergic synapses. Earlier in development, there are dramatic changes in the number and distribution of GABAergic and glutamatergic neurons, which produce profound alterations in synaptic transmission. However, little is known how the E/I ratio is dynamically adjusted through the postnatal critical periods, when the strength of excitatory and inhibitory synapses are rapidly changing.

The mediodorsal thalamus (MD) represents a fundamental subcortical relay to the prefrontal cortex (PFC), and is thought to be highly implicated in modulation of cognitive performance. Additionally, it undergoes highly conserved developmental stages, which, when dysregulated, can have detrimental consequences.

Within this line of evidence, we have recently elucidated the role played by thalamic afferents on the morphological organization and the functioning of the PFC and the amygdala. We hypothesized long-term altered morphology, neuronal activation and disrupted GABAergic neurotransmission in both structures as a consequence of early onset of MD damage.

Early MD lesion-based research has firmly established that early disruption of thalamic inputs to frontal cortical-amygdala circuits alters the normal development of excitatory and inhibitory neurotransmission, which is critical for the acquisition of mature brain function emerging during late adolescence. In this respect, the clear capacity of early developmental insult of the MD to change gene expression, neural function and morphology in multiple brain regions must be interpreted as a cautionary tale that cross-sectional genetic manipulations alone may have difficulty capturing the full picture of disease process and mechanism in mental disorders.

S44.3
DISRUPTION OF CORTICOTHALAMIC SYSTEMS IN A MODEL OF FIRST-EPISODE PSYCHOSIS

Didier Pinault

INSERM U1114, Neuropsychologie cognitive et physiopathologie de la schizophrénie, Strasbourg, France; FMTS, Faculté de médecine, Université de Strasbourg, Strasbourg, France

Schizophrenia is a progressive, debilitating mental illness characterized by a loss of contact with reality, personality disorders, mood symptoms, sensorimotor and cognitive impairments. There is increasing evidence that this mental illness is associated with abnormal anatomo-functional connectivities in corticothalamic systems at all stages of schizophrenia, including the prodromal phase and in high-risk mental health patients for psychosis. Cortical gamma frequency (30–80 Hz) oscillations, naturally implicated in global brain operations, are excessively amplified not only during hallucinations but also in high-risk patients who later develop psychosis. The mechanisms underlying the pathogenesis of schizophrenia-related brain networks dysfunction are unknown. Inour laboratory, an abnormally excessive amplification of spontaneously-occurring gamma oscillations is recorded in the somatosensory corticothalamic system of the adult rat under the acute influence of ketamine, a non-competitive antagonist of glutamate NMDA receptors and a psychotomimetic substance that mimics the effects of first-episode schizophrenia in healthy humans. Furthermore, this ketamine-induced excessive ongoing gamma oscillations disturb sensory-related cortical and thalamic gamma oscillations. In conclusion, our results show, for the first time, that ketamine reduces the corticothalamic system's ability to encode/integrate information, allowing us to posit pathophysiological hypotheses regarding sensory and cognitive disorders in patients with schizophrenia.

S44.4
THALAMOCORTICAL INTERACTIONS IMPORTANT FOR COGNITIVE FUNCTIONS: THE ROLE OF THE MEDIODORSAL THALAMUS

Anna Mitchell
Department of Experimental Psychology, University of Oxford, UK

Distributed neural networks across the brain govern cognitive processes and guide our behavioural outputs. Typically, cortical structures including the temporal lobes are investigated in these cognitive processes. Recent behavioural and cognitive neuroscience evidence from my laboratory demonstrates that communication between the cortex and mediodorsal thalamus supports the ability of primates to learn rapidly and make dynamic choices under changing circumstances. This behavioural evidence demonstrates that the mediodorsal thalamus actively contributes to information processing, complementing the contribution of the frontal cortex itself. Analyses documenting the resting state functional neuroimaging of the primate brain after circumscribed damage to the mediodorsal thalamic will also be presented.

S45 Room: Ballroom
THE BRAIN NETWORK OF LEVODOPA-INDUCED DYSKINESIA IN PARKINSON'S DISEASE

Chair: Salvatore Galati, CH

S45.1
SLEEP-DEPENDENT PLASTICITY IN THE EMERGING OF LEVODOPA-INDUCED DYSKINESIAS

Salvatore Galati
Neurocenter of Southern Switzerland (NSI), Lugano, Switzerland

Homeostatic adjustments of network excitability occurring during sleep has been proved to be crucial for reducing plasticity thresholds and recent evidence in animal models shown that an impaired synaptic homeostasis (SH) underlies the appearance of levodopa-induced dyskinesia (LID). We are extending these results in human disease by an all-night high-density EEG (hd-EEG) study performed at different disease duration.

We found that there was a difference between normal subjects and PD patients in terms of the physiological reduction SWA power, i.e. synaptic strength. Moreover, we also found a difference within the three groups of the same parameter suggesting a not adequate synaptic down-scaling during NREM sleep in patients with dyskinesia. Our results are consistent with an impaired SH in patients with PD that is more pronounced in those patients with dyskinesia.

S45.2
IN VIVO ELECTROPHYSIOLOGY OF LEVODOPA-INDUCED DYSKINESIAS

Alessandro Stefani
University of Rome Tor Vergata, Italy

In order to develop more effective treatment for Levodopa-induced dyskinesia (LIDs), in Parkinson's disease (PD) patients, the scientific community is devoted to collect original electrophysiological data either in mammalian PD models or in subject undergoing stereotactic surgery.

Recent literature (and initial investments in unconventional approach such as transcranial direct current stimulation) is indicating that the original a-priori, by which LIDs were merely attributable to the lack of synaptic de-potentiation inside striatum, should be revised. We are aware that Parkinsonian rodents loose bidirectional plasticity (as shown by Thiele et al. 2014, by applying spike-timing dependent protocols to corticostriatal synapses); they revealed that, following lesioning, the indirect pathway only exhibited LTP, whereas the direct pathway only showed LTD. However, extrastriatal regions partecipate as well to the pathogenesis of LIDs. The remodelling of the so called hyperdirect pathway, as a "plausible neural substrate for understanding the rTMS-related effects on PD patients with LIDs" is a clear example. Further, the low frequency magnetic stimulation of cerbellum, affecting dentato-fugal pathways, provided a transient but not negligible relief from involuntary movements. As elegantly posed by Calabresi and co-authors, "hyperkinetic disorders are all characterized" not only "by loss of the ability to reverse synaptic plasticity" but also to "an associated increase in the excitability of excitatory neuronal inputs to a range of cortical and subcortical brain areas".

In addition, the success of deep brain stimulation (DBS) onto the subthalamic nucleus (STN), or even STN microlesioning, are linked to the beneficial modulation of endogenous band frequencies, shared in synchrony by subcortical and cortical regions, and encoding movement performance. Several experimental lines (and evidence from the surgery room) suggest that, in PD, basal ganglia stations bear an "excessive" beta band (βB), which correlates with major hypokinetic signs in PD; less clear is the pathogenetic role played by lower or higher band frequency. In principle, gamma (γ) oscillations were usually considered as simply pro-kinetic; yet, our experience with in-vivo recordings in freely moving rats following 6-OHDA denervation, show that the development of LID is characterized by A) a slight increase in the cumulative power of βB and, notably B) a

significant large augmentation in the γB frequency (60–80 Hz). This latter effect reaches a plateau in the frontal cortex (to note, bilaterally) and the left globus pallidus after the second week of LD treatment. The relevance of these findings is intuitive, as far as the new-called adaptive DBS, based upon closed-loop changing of stimulation parameters according to the patient's clinical state, promises to deliver appropriate and timing protocols (provided the consistent acquisition of electrophysiological biomarkers).

Our contribution to the symposium will recapitulate the available literature on striatal and extra-striatal electrophysiological features in correlation with the occurrence and severity of dyskinesia; the circuitry mechanisms underlying the development of LIDs; and the specific therapeutic roles sub-served either by DBS or dopaminergic agents.

S45.3
DOPAMINE-MEDIATED METABOLISM OF CAMP/CGMP AND THE LEVODOPA-INDUCED DYSKINESIAS

Vincenza D'Angelo
University of Rome Tor Vergata, Italy

Levodopa (l-dopa)-induced dyskinesia (LIDs) features almost all patients with Parkinson's disease (PD), with a severe impact on their quality of life. The arsenal of pharmacotherapies, including alterative formulations of levodopa (LD), has improved recently. Some trials, i.e. with new extended-release amantadine, the serotonin agent eltoprazine or the glutamate mGluR5 modulator mavoglurant appeared promising. Yet, their translation to clinical practice remains challenging; and surprisingly scarse are the investments in studying pathways profoundly involved in the endogenous catabolism of the dopamine signaling in the basal ganglia (BG), such as the activity of guanylate cyclases (which governs the production of cyclic guanosyn monophosphate – cGMP -) and endogenous phosphodiesterases (PDE). Inferred a decade ago in PD patients undergoing deep brain stimulation the possible role of cGMP in shaping motor perfomance was reinforced by the description of a novel from of striatal plasticity, based upon the interplay between nitric-oxide (NO) interneurons and medium spiny neurons. To note, LID associate with an upregulation in the number of nNOS-expressing interneurons and the neuronal NOS inhibitor 7-nitroindazole exerts anti-dyskinetic effects in rodents.

Some of our observations in this field may be summarized as it follows: in the 6-hydroxydopamine (6-OHDA) model, after chronic LD, cAMP and cGMP are differentially regulated in eukinetic animals: the cAMP level increases in the cortex and striatum but decreases in the globus pallidus, whereas cGMP decreases below baseline levels in the contralateral cortico-striatal- pallidal regions. At contrast, in dyskinetic animals, we found an absolute decrease in cAMP and cGMP levels in cortico-striatal- pallidal regions of both hemispheres, sensitive to the pretreatment with the aspecific PDE antagonist zaprinast, coherent with a putative increased catabolism through PDE overactivity. PDE10A is the most abundant PDE in BG, expressed as a dual substrate enzyme, regulating cAMP and cGMP cascades. PDE10A mRNA levels were specifically decreased in striatal neurons (non in accumbens) at 10 weeks after 6-OHDA. In parallel, PDE10A protein levels and activity decreased in both striato-pallidal and striato-nigral projections.

Further experiments (microdialysis assays and tissue homogenates of the striatum, collected timingly during the LD challenge) suggested that extracellular and intracellular cAMP/cGMP levels were lower during the increasing phase of LIDs, whilst cAMP/cGMP levels increased during the phase of decreasing and extinction of dyskinesias. The LD-induced lowering of striatal cGMP and cAMP was prevented by pretreatment with the multipotent drug amantadine (active on PDE asides from NMDA channels). In addition, higher striatal hydrolyzing cGMP-PDE (and not hydrolyzing cAMP-PDE activity) featured dyskinetic animals; hence, only low cGMP, as far as acute LIDs develop, is due to increased catabolism.

The translational value of these findings is questionable. It was shown that both zaprinast and UK-343664 were able "to rescue the induction of LTD via a mechanism requiring the modulation of intracellular cGMP"; consistently, higher dyskinesia scores correlated with lower 11C-IMA107 BPND binding (imaging marker of PDE10 expression) in striatum and pallidum of advanced PD patients. However, far from established is whether these abnormalities have a pathogenetic role or, instead, represent compensatory mechanisms. Indeed, Sancesario showed that the expressions of the isozyme phosphodiesterase-1B and -10A were not changed after LD. New investigations are welcome in order to clarify the complex interplay between excitatory transmission (cholinergic and glutamatergic) and NO-PDE cascades in BG circuitries during well-defined behavioral states.

S45.4
OPIOIDERGIC SYSTEM AS A POTENTIAL TARGET FOR PREVENTING LEVODOPA-INDUCED DYSKINESIAS?

Alain Kaelin
Neurocenter of Southern Switzerland, Lugano, Switzerland

Several different neurotransmitter systems have been linked with levodopa-induced dyskinesia in the par-

kinsonian striatum beside dopamine. However, all new drug treatments targeting these non-dopaminergic neuromodulatory systems have failed. Several large-scale multicentre clinical studies were prematurely stopped because of lack of efficacy or increase in parkinsonian symptoms. For example, trials with serotoninergic agonists or metabotropic glutamate receptors antagonists did not show the expected efficacy. However, patients presenting with severe dyskinesia were usually included in these studies, and only the direct symptomatic effect of the drug was investigated. In contrast, strategies trying to reduce the risk of developing levodopa-induced dyskinesia by acting on non-dopaminergic pathways at an earlier stage or even before starting levodopa therapy have been neglected. The opioidergic striatal neuropeptides are also a profound modulator of the dopaminergic system and act through several specific receptors in and outside the striatum. This opioidergic system shows profound changes in the parkinsonian brain already before any dopaminergic treatment. We suggest that this system is heavily involved in the sensitization of the postsynaptic dopaminergic pathways after dopaminergic denervation, the so-called "priming" responsible for the induction of levodopa-induced dyskinesia. We will review newest evidences about the role of the opioidergic system in striatal physiology and discuss possible new therapeutic opioidergic targets to avoid the "priming" phenomenon.

S45.5
THE ROLE OF CEREBELLUM IN LEVODOPA-INDUCED DYSKINESIAS

Giacomo Koch
Non invasive Brain Stimulation Unit, Santa Lucia Foundation IRRCS, Rome, Italy

understood. Despite the classical view that LID might be generated exclusively by the disinhibition of cortical motor areas secondary to abnormal output from the basal ganglia in the striato–thalamo-cortical circuit, there is indirect evidence that the cerebellum may play a role in LIDs.

Repetitive transcranial magnetic stimulation (rTMS) can be utilized to influence the excitability of the cerebral cortex, providing important insights into mechanisms of cortical plasticity. When employed to induce long-lasting changes in the excitability of synapses, rTMS can be seen as a therapeutic tool in several neurological and psychiatric conditions. rTMS is important for investigating the functions of cerebellar plasticity, in particular interacting with the cerebello-thalamo- cortical pathway.

In my presentation I will provide an overview of recent works showing that rTMS applied over the cerebellum induces long-lasting changes in the excitability of the interconnected primary motor cortex and parieto-frontal networks, through the modulation of cerebello-thalamo-cortical pathways. In patients with PD, with mild to moderate LID, repeated sessions of bilateral cerebellar inhibitory stimulation after regular doses of levodopa induce a sustained reduction of dyskinesia. Non-invasive cerebellar stimulation might help to re-establish the cerebellar and basal ganglia control over the non-salient inputs to the motor areas during synaptic dopaminergic surges.

The high potential of cerebellar rTMS as a therapeutic tool in PD patients with LIDs could depend on the possibility of modulating several interconnected remote areas, through the activation of different systems, such as the cerebello-thalamo- cortical networks.

S46 Room: Marie Louise 2
THE KEY ROLE OF CATECHOLAMINES IN STRESS, ADDICTION AND HUNTINGTON'S DISEASE

Chairs: Giulia Fois, FR and François Georges, FR

S46.1
BEHAVIOURAL AND CELLULAR INSIGHTS INTO NICOTINE AND STRESS INTERPLAY

Sebastian Fernandez
Team Molecular mechanisms of neuronal plasticity in health and disease Institut de Pharmacologie Moléculaire et Cellulaire 660 Route des Lucioles Sophia Antipolis 06560 VALBONNE France

Nicotine dependence is the leading cause of illness and premature death in patients with psychiatric disorders. Smokers who successfully abstain are at increased risk of experiencing a depressive episode when compared to individuals who continue smoking. More severe drug withdrawal episodes have also been reported in people with a history of depression. Comorbidity, which is defined by the co-occurrence of mental and substance use disorders, is much more common than expected and may be underpinned by common cellular adaptations within shared neuronal brain circuits. Here, we will highlight the intertwined relationship between stress, a key environmental risk factor that facilitates mood disorders, and nicotine, the main psychoactive constituent of tobacco. We will present evidence for such an interplay focusing on the enduring dysregulation within the reward dopamine system.

S46.2
FUNCTIONAL REMODELING OF GLUTAMATERGIC INPUTS TO LOCUS COERULEUS NEURONS

DURING THE ADOLESCENCE TO ADULTHOOD TRANSITION

Raffaella Tonini
Neuroscience and Brain Technologies Department, Istituto Italiano di Tecnologia, Genova, Italy

S46.3
CONTEXT-SWITCH PROMOTES CHANGES IN THE TONIC AND INTEGRATIVE ELECTROPHYSIOLOGICAL PROPERTIES OF VTA DOPAMINERGIC NEURONS

Giulia Fois
Université de Bordeaux, Centre National de la Recherche Scientifique, Neurodegeneratives Diseases Institute, UMR 5293, F-33076 Bordeaux, France

A key feature of human and animal behavior is to learn from environmental stimuli to efficiently adapt. The ability to learn about a new environment and to minimize contact with aversive experience is a hallmark of adaptive behavior and recent studies have demonstrated a key role of the ventral tegmental area (VTA) dopamine neurons in this process. Moreover, a critical role in the integration of relevant contextual information to modulate goal-directed behaviors is played by the hippocampus, which participates in control on the activity of dopamine neurons in VTA. More specifically, we have previously reported that the ventral subiculum of hippocampus (vSUB) regulate VTA dopamine neurons activity through the Bed nucleus of stria terminalis (BNST), and the potentiation of vSUB-BNST- VTA pathway increase locomotor activity induced by a subthreshold dose of cocaine. Also, we know that BNST is a key structure controlling negative emotional states, and it integrates diverse inputs from vSUB and infralimbic cortex to modulate anxiety. On the other hands, it has been showed a role of dorsal hippocampus (CA3)-later septum-VTA pathway on modulation of cocaine-seeking behaviour by contextual stimuli. Therefore, these transsynaptic links between hippocampus and VTA appear to be important substrates by which environmental context regulates goal-directed behaviour and anxiety. Here we investigate how stress and novelty induced by a switch of context trigger changes in the tonic and integrative electrophysiological properties of VTA dopamine neurons, and dissect the neuronal circuits governing the activity of the VTA dopamine neurons when animals are exposed to a new environmental context.

S46.4
SYNAPTIC DYSFUNCTION IN EARLY SYMPTOMATIC STAGE IN A MOUSE MODEL OF HUNTINGTON'S DISEASE

Christelle Glangetas
Department of Basic Neurosciences, University of Geneva, Switzerland.

Huntington's disease (HD) is an inherited, progressive neurodegenerative disorder characterized by motor, cognitive and psychiatric dysfunctions. The disease is caused by a mutation of the Huntingtin gene. Although many symptoms of HD are associated to neuronal death of striatal neurons, this degeneration is preceded by synaptic dysfunctions. The molecular mechanisms that lead to these early synaptic dysfunctions and how aberrant plasticity at this stage contributes to the development of behavioral traits in HD are still open questions.

Previous studies have shown that non-canonical NMDARs containing GluN3A subunits are aberrantly expressed in HD mouse models in the striatum. These non-canonical NMDARs have unique properties and therefore confer to synapses distinctive properties that may affect the induction and the expression of synaptic plasticity. Using the yeast artificial chromosome expressing the mutant Huntingtin mouse model (YAC128) and cutting-edge techniques on electrophysiology associated with optogenetic tools, I will discuss synaptic properties of glutamatergic transmission in a cell-type specific and input specific manner as well as the induction and expression of synaptic plasticity at excitatory inputs onto medium spiny neurons (MSNs). Identifying the mechanisms that regulate synaptic dysfunctions in a HD mouse model will help to design specific and targeted pharmacological interventions that could rescue and delay or even prevent the onset of the disease.

S47 Room: Clermont Suite
PHYSIOPATHOLOGICAL MECHANISMS OF AIRWAY PROTECTION: COUGH AND SWALLOW REFLEXES

Chair: Christian Gestreau, FA

S47.1
ROLE OF THE DORSAL MEDULLA IN THE NEUROGENESIS OF COUGH

Donald Bolser
College of Veterinary Medicine, University of Florida, USA

The dorsal medulla, mainly in the region of the nucleus of the tractus solitarius (NTS) has primarily been viewed as a control region for the cough reflex that has minimal complexity. Current understanding of the role of the NTS is limited to the existence of second order interneurons which receive afferent input from vagal cough related afferents and transmit this information to other brainstem regions. Our objective was to perturb cough

with a single intervention in the NTS. We speculated that the NTS may have a more complex role in the expression of coughing than is supported by current hypotheses. We utilized microinjections of the excitatory amino acid antagonist, kynurenic acid (KA), into the rostral or caudal NTS to perturb cough in anesthetized cats. Bilateral microinjections of 100 mM KA (total approximately 100 nL) into the rostral NTS (0.8 and 1.6 mm rostral to obex) induced suppression of tracheobronchial coughing elicited by mechanical stimulation of the intrathoracic airway. Suppression of cough was associated with significant prolongation of the cough inspiratory phase duration and total cough cycle time, including cough apneusis/apraxia. We also observed significant co-activation of inspiratory and expiratory muscles during cough. Microinjections of KA into the caudal NTS (0.8 and 1.6 mm caudal to obex) had no effect on cough. Antitussive drugs do not disrupt the temporal features of coughing, suggesting that KA acted in these experiments on neural elements that are insensitive to centrally-acting cough suppressants. Our results suggest that neurons in the region of the NTS have a role in controlling phase durations for coughing. Further, excitatory amino acid neurotransmission in the caudal NTS is not critical for cough in the anesthetized cat. Supported by NIH R01 103415.

S47.2
PERIPHERAL AND CENTRAL MODULATION OF TRACHEOBRONCHIAL COUGH

Ivan Poliacek
Comenius University in Bratislava, Jessenius Faculty of Medicine in Martin, Institute of Medical Biophysics, Martin, Slovakia

Cough is modulated by changes in the cough primary afferent drive, the peripheral signaling and feedback, the performance of central control circuits and simultaneous occurrence of other behaviors. The complexity and non-linear appearance of cough control mechanisms likely produces disproportional cough attenuation due to reduced vagal conductivity. Among peripheral stimuli, volume feedback has a limited effect on cough, however, irregular cough volumes/airflows induced changes in spatio-temporal parameters of cough that were similar to those during breathing. When multiple behaviors are induced in close temporal proximity, the complex and rhythmic responses of sneezing and swallowing preferentially occur between successive coughs. Abrupt non-rhythmic behaviors such as aspiration and expiration reflex superimpose over cough motor bursts with no significant alterations of the cough spatio-temporal features. The prior occurrence of aspiration reflexes or swallowing reduces cough responsiveness and/or increases cough latencies. Local lesions, inhibition or excitation of neuronal activity within the brainstem attenuate coughing. Alterations of the temporal features of cough are mostly produced by peripheral and not central modulation of coughing. Cough motor drive increases with induction of aspiration reflex during the cough inspiratory phase, when swallow, sneeze or expiration reflex are executed concurrently with coughing and with simultaneous tracheobronchial and laryngeal stimulation. Irritation of the airways exaggerates cough, however, subthreshold mechanical stimuli do not modulate tracheobronchial cough. Shared neuronal components and changes in neuronal excitability are likely employed in the modulation of cough. Complex central interactions, sequencing of airway motor acts and peripheral stimuli may result in the disruption of coughing or in the enhancement of cough. Some of the stimulation paradigms that alter cough excitability in animal models might correct inappropriate or excessive coughing in humans. Neither behavioral, nor pharmacological and combined modulation of coughing is sufficiently utilized in current medical practice.

S47.3
NEW INSIGHTS INTO SWALLOW-BREATHING COORDINATION

Kofi-Kermit Horton
Department of Molecular Pharmacology and Physiology, Morsani College of Medicine, University of South Florida, Tampa, Florida, USA

Breathing and swallowing are closely related in their central control and are highly coordinated. Despite extensive studies, mechanisms of swallow-breathing coordination remain unclear. Here, we postulated that dynamic processes in brainstem networks relying on peripheral afferent feedback and interactions between central pattern generators (CPGs) contribute to swallow-breathing coordination. Electrical stimulation of the superior laryngeal nerves (SLN) or water injected in the pharyngeal cavity was used to trigger swallows in decerebrate, paralyzed and artificially ventilated cats while motor activities were recorded from phrenic, hypoglossal and vagal nerves. Mechanical ventilation was either triggered mostly in phase with central inspiratory activity (phrenic-driven mode, PD) or set independently from the phrenic activity (free-run mode, FR) to alter the timing of bronchopulmonary vagal afferent input and allow analysis of its influence on swallow-breathing coordination and swallowing parameters. Results showed significant differences in phase relationships between breathing and swallowing according to the type of stimulus (SLN vs. Water) and the type of ventilation (PD vs. FR). When repetitive swallows and apnea were induced by long bouts of SLN-stimulation in FR, we observed an altered frequency distribution of swallows

within the ventilation cycle, with fewer swallows during lung inflation, suggesting an inhibition from vagal afferent inputs on swallow CPG. Also, results of in-depth analyses of the temporal relationship between central respiratory activity and swallowing suggested reciprocal inhibitory connections between the two CPGs. We conclude that both peripheral and central mechanisms of gating are determinants for swallow-breathing coordination and swallow dynamics. These observations may be relevant for the management of health and nutritional outcomes of dysphagic patients and patients with pulmonary disorders.

S47.4
BRAINSTEM-MEDIATED CONTROL OF PROTECTIVE UPPER AIRWAY REFLEXES AND COORDINATION WITH BREATHING

Tara Bautista
Respiratory Neurophysiology laboratory, Systems Neurophysiology division, Florey Institute of Neuroscience and Mental Health, Parkville, Melbourne, VIC Australia

The upper airways are not merely conductance passages for both respiratory and digestive tracts; they play an imperative role in maintaining the integrity of the lower airways. Cranial motoneurons innervating the upper airway are located in the brainstem. Accordingly, the basic neural circuits subserving protective upper airway reflexes, including those associated with reflex swallowing, are also located within the brainstem. Previous research has also shown the role of medullary nuclei in mediating the coordination of reflex swallowing and breathing. Such nuclei include the Bötzinger complex and the *nucleus tractus solitariius*. Here I will describe recent evidence in rodent models that point to the critical involvement of the Kölliker-Fuse nucleus (KF) within the dorsolateral pons in the pre-motor control of the upper airways and protective upper airway reflexes. The KF is required in a number of aspects associated with reflex swallowing, particularly laryngeal adduction, breath-hold, gating of swallows and timing of swallows in relation to the respiratory cycle. KF neurons have recently been found to express Forkhead Box Protein 2 (FOXP2), a transcription factor implicated in vocalization and language development. The significance of these novel findings will be described in the context of upper airway dysfunction in human disorders.

S47.5
CHANGES IN CORTICAL OROPHARYNGEAL SENSORY INTEGRATION IN PARKINSON'S DISEASE

Teresa Pitts
Kentucky Spinal Cord Injury Research Center University of Louisville, USA

Movement of a bolus from the mouth into the pharynx activates sensory mechanoreceptors. Using electroencephalography, somatosensory cortical-evoked potentials resulting from pharyngeal mechanical stimulation (PSEP) have been studied in young healthy individuals. However, limited information is known about changes in processing of oropharyngeal afferent signals with Parkinson's disease (PD). To determine if there are changes to the PSEP 13 persons with Parkinson's disease (PD) and 7 older adults (HOA) were tested with two stimuli (S1-first; S2-second) to the oropharyngeal wall delivered 500 ms apart. Results demonstrated PSEP P1, N1, and P2 component peaks were present in all participants, and the N2 peak was present in 17/20 participants. However, the PD participants had a decreased N2 latency and gated the P1, P2, and N2 responses (S2/S1 under 0.6). Compared to the HOAs, the PD participants had greater evidence of gating the P1 and N2 component peaks. These results suggest that persons with PD have significant changes in their sensory processing of pharyngeal mechanical stimulation. In conclusion, the altered processing of sensory feedback from the pharynx may contribute to disordered swallow in patients with PD.

S48 Room: Marie Louise 1
BRIDGING THE GAP IN ADDICTION RESEARCH: CLINICAL STUDIES IN THE FIELD OF THE NOVEL PSYCHOACTIVE SUBSTANCES

Chair: Laura Orsolini, IT

S48.1
THE 'ENDLESS TRIP': PSYCHOPATHOLOGICAL AND CLINICAL ISSUES RELATED TO THE NEW SYNTHETIC HALLUCINOGENS

Laura Orsolini
Department of Psychopharmacology, Drug Abuse and Novel Psychoactive Substances Research Unit – University of Hertfordshire, Hatfield, UK; EFPT working group on Psychoactive Substance Use Disorders; Department of Psychiatry and Neuropsychology, Maastricht University, Maastricht, Netherlands

The class of hallucinogens comprises all drugs able to alter consciousness by both distorting the mode of user's perception of time, motion, colour, sounds and self and by inducing sensory and perceptual disturbances. Hallucinogens are also called *'psychedelics'* (a term also describing the 'classical hallucinogen' such as LSD [N,N-diethyl-D-lysergamide] and psilocybin), *'psychotomimetics'* (a term emphasizing their effects that mimic psychotic symptoms) and *'entheogens'* (due to

the mystical-type experiences these drugs may induce). In general, the term classical *'hallucinogen'* is used to connote all drugs acting as agonists at the 5-HT_{2A} receptor. Beyond these, we include the *'synthetic hallucinogens'*, mostly belonging to the NPS category, e.g. lysergamides (i.e. AL-LAD, 1P-LSD, etc.), synthetic tryptamines (i.e. 5-MeO-DALT, DiPT, 5-Meo-DiPT, etc.) and synthetic phenethylamines (i.e. 2C-series and their derivatives, DOX series, etc.). New psychopathological and clinical issues are emerging after the capillary and alarming dissemination of these new synthetic drugs, i.e. the HPPD (Hallucinogen Persisting Perception Disorder) a syndrome characterized by prolonged or reoccurring perceptual symptoms, reminiscent of acute hallucinogen effects. HPPD was associated with a broader range of LSD -like substances, including cannabis, MDMA (methylenedioxymethamphetamine), psilocybin, mescaline and also, recently, with the last newest synthetic hallucinogens. A critical overview on clinical and psychopathological secondary to synthetic/new hallucinogenic/psychedelic drugs will be here discusses, also by deepening the most effective and safest pharmacological treatment options so far available.

S48.2
FROM CANNABIS PSYCHOSIS TO SPICEOPHRENIA

Duccio Papanti

'Psychopharmacology; Drug Misuse; and Novel Psychoactive Substances' Research Unit; School of Life and Medical Sciences, University of Hertfordshire, Hatfield, Herts, UK

Synthetic cannabinoids (SC) are cannabimimetic designer compounds found in so called 'Spice drugs' which represent a large and diverse class of Novel Psychoactive Substances (NPS). SC can exert full, or even super-agonist, activity at cannabinoid receptors (CB-rs), while tetrahydrocannabinol (THC), the main euphoriant phytocannabinoid present in cannabis, has a weak, partial agonism, activity at CB-rs. SC may therefore present with higher addictive liablility levels than cannabis. Spice consumption has been associated with schizophrenomimetic effects, the induction of psychotic disorders, and clinically relevant levels of acute toxicity. These conditions are qualitatively and quantitatively different from those observed after cannabis intake.

S48.3
HARM REDUCTION OF NOVEL PSYCHOACTIVE SUBSTANCES

Levente Móró

Department of Behavioural Sciences and Philosophy, Faculty of Social Sciences, University of Turku, Finland

The rapid emergence of Novel Psychoactive Substances (NPS) has significantly reshaped the global drug scene, inducing fundamental changes in markets, policies, and drug user subcultures. In order to understand the still-ongoing NPS phenomenon, studies must characterise its few similarities and many differences compared to traditional drugs, and explain the terminology and appeal of new drugs. These recent changes pose new challenges also for drug-related harm reduction work. From the drug users' perspectives, NPS-related harms are derived from set and setting. These harms could be identified by analysing scientific literature, observing drug discussion groups, monitoring warnings about drug-related emergencies, and following legislative changes. Some of these harms are, e.g., drug misidentification, mixtures, naming, (over)dosing, interactions, scams, legal consequences, psychosocial risks, etc. In my presentation, I describe some currently operating NPS harm reduction measures and best-practices – e.g., drug checking services, public databases, and information dissemination – with European case examples, including also drug user self-help activities. Moreover, I discuss harm reduction at parties/festivals, market force involvement, public media regulation, consumer protection of NPS users, and promotion of a "smarter" drug user culture. The conclusion that logically follows from the above analyses (of NPS-related harms, harm reduction best-practices, and needed policies) is that the lifecycles of drugs cannot be stopped by force, but their courses may be partially affected by carefully chosen policies and interventions. For the long-term goal of establishing evidence-based and integrated psychoactive substance policies that also reduce use related harm, changes are urgently needed.

S49 Room: Reading Room
ROLE OF UBIQUITIN PROTEASOME SYSTEM (UPS) IN SYNAPTIC PLASTICITY: IMPLICATIONS FOR NEW THERAPEUTIC APPROACHES

Chair: Patrizia Romualdi, IT

S49.1
INVOLVEMENT OF PROTEASOME MACHINERY IN PAIN AND ADDICTION

Francesca Felicia Caputi

Dept. of Pharmacy and Biotechnology, Alma Mater Studiorum - University of Bologna, via Irnerio 48, 40126 Bologna, Italy

The Ubiquitin-26S Proteasome System (UPS) is the major route used by eukaryotic cells for the disposal of misfolded or damaged proteins and for controlling pro-

tein lifespan. As a consequence, UPS regulates fundamental cellular processes such as protein quality control, DNA repair, or signal transduction. Due to its involvement in many intracellular pathways, the 26S proteasome has emerged as a significant drug target in various disease. Several studies dealing with drugs of abuse showed that the proteasome subunits, or proteins involved in the ubiquitination process, are often regulated by psychoactive substances. Notably, the UPS-mediated degradation system plays a crucial role in synaptic remodeling and in memory reconsolidation mechanisms after cocaine exposure; in addition, UPS is also involved in ethanol (EtOH)-induced dysfunctions affecting transcriptional factors turnover and epigenetic mechanisms. In regard to cocaine and EtOH, recent data from our laboratory demonstrated that they caused an opposite modulation of the β5 chymotrypsin-like activity in neuroblastoma cells. Indeed, cocaine increased the proteolytic activity (131 ± 10.63 vs control 100 ± 4.54, $p = 0.004$), whereas ethanol induced a significant reduction (86 ± 1.96 vs control 100 ± 2.85, $p = 0.031$). We also highlighted selected gene expression alterations of specific 26S proteasome subunits belonging to the 20S core particle. In particular, the prevalent up-regulation of the β1 subunit recorded after cocaine (1.50 ± 0.07 vs control 1.00 ± 0.04, $p = 0.0008$) and EtOH (1.59 ± 0.05 vs control 1.00 ± 0.04, $p < 0.001$) treatments, as well as the analogous increase of the α5 subunit, may suggest that the oxidative stress conditions promoted by the two substances require the assembling of new functional proteasome complexes. Furthermore, other psychoactive substances seem to act on the UPS machinery. In this regard it has been proposed that the higher toxicity of methamphetamine (METH) compared to cocaine could be due to its longer inhibitory effect on proteasome activity characterized by caspase-dependent apoptosis and damaged protein accumulation. However, the contribution of METH to UPS dysfunction has not been sufficiently clarified. Proteasomal dysfunction has been also related to neuropathic pain condition and management. In fact, the intrathecal administration of distinct proteasome inhibitors attenuated hyperalgesia and allodynia in neuropathic rats suggesting that the UPS-mediated protein degradation is required for maintenance of chronic pain. In addition, the co-administration of the proteasome inhibitor MG-132 with morphine prevents the development of morphine tolerance. Finally, recent data showed that ibuprofen increases the aggregation of misfolded ubiquitylated protein and that aspirin seems to promote apoptosis through the inhibition of proteasome function. Therefore diverse pathological conditions, such as drug addiction or chronic neuropathic pain, may affect the abundance of different UPS key subunits and/or the UPS proteolytic activity thus strengthening the hypothesis that despite their differences the UPS machinery could represent a common molecular target.

S49.2
NEUROINFLAMMATION, AUTOPHAGY AND PROTEASOME

Diego Ruano Caballero

Instituto de Biomedicina de Sevilla (IBiS)-Hospital Universitario Virgen del Rocío/Consejo Superior de Investigaciones Científicas/Universidad de Sevilla, 41013-Sevilla, Spain; Departamento de Bioquímica y Biología Molecular, Facultad de Farmacia, Universidad de Sevilla, 41012-Sevilla, Spain

Proteostasis alteration and neuroinflammation are characteristic features of aged hippocampus. Cellular proteostasis is maintained by molecular chaperones and the two major proteolytic systems such as the ubiquitin proteasome system and the autophagy lysosomal pathway. On the other hand, neuroinflammation is characterized by microglia activation and pro-inflammatory cytokines production. The most relevant age-related modifications concerning proteostasis and neuroinflammation in rat hippocampus are: 1) the presence of active microglial cells together with TNFα and IL-1β expression; 2) higher content of immunoproteasomes; 3) decreased proteasomal activity; 4) autophagy malfunction; 5) ERAD disruption and 5) higher susceptibility to neurodegeneration under cellular stress. Here, we show that age-related neuroinflammation might be in the base of all of the hippocampal proteostasis alterations that occur in aged rats. Indeed, using an *in vivo* model of hippocampal neuroinflammation we are able to reproduce all these alterations. Taking together, these data allow us to propose that a reduction in the number of neuroinflammatory events along the lifespan could result in lower neurodegeneration, delaying then the onset of the age-related neurodegenerative diseases and increasing the healthspan and success aging.

S49.3
AGING RELATED DISEASES AND UBIQUITINE PROTEASOME

Niki Chondrogianni

Institute of Biology, Medicinal Chemistry and Biotechnology, National Hellenic Research Foundation, Greece

Proteasomes are constituents of the cellular proteolytic networks that maintain protein homeostasis through regulated proteolysis of normal and abnormal (in any way) proteins. Proteasome activation in cell lines has been shown to result to cellular lifespan extension and to exert protein anti-aggregation activity. Using *Caenorhabditis elegans* as a model, we analyzed in detail the proteasome status upon the progression of aging

and Alzheimer's disease (AD) and we investigated the effects of enhanced proteasome activities on the progression of the above mentioned phenomena. The obtained results were validated in human and murine cells of neuronal origin. Proteasome activation in *C. elegans* either through genetic means or through compounds resulted in enhanced levels of proteasome activities that led to a SKN-1- and proteasome activation-dependent lifespan extension. The elevated proteasome function confered lower paralysis rates in various AD nematode models accompanied by decreased Aβ deposits thus ultimately decelerating the progression of AD phenotype. More importantly, similar positive results were also delivered in human neuroblastoma cells and in murine cortical neurons. Our results suggest that proteasome activation with downstream positive outcomes on aging and AD, an aggregation-related disease, is feasible in both a genetic and a non-genetic manipulation manner in a multicellular organism. Moreover they unveil the need for identification of anti-aging and anti-amyloidogenic compounds among the nutrients found in our normal diet.

S50 Room: Marie Louise 1
BRAIN DIRECTED GENE TRANSFER STRATEGIES. WHAT WE LEARNED FROM PRECLINICAL RESEARCH FOR RARE DISEASES

Chairs: Giuseppe Ronzitti, FR and Fatima Bosch, ES

S50.1
DOPAMINE TRANSPORTER GENE TRANSFER IN DAT-KO MICE AS A THERAPEUTIC STRATEGY FOR DTDS

Stefano Espinoza
Istituto Italiano di Tecnologia Department of Neuroscience and Brain Technologies, Fondazione Istituto Italiano di Tecnologia, 16163 Genova, Italy

Parkinson's Disease (PD) is a neurodegenerative disorder characterized by the loss of dopaminergic neurons of the substantia nigra pars compacta, and by the presence of intra-neuronal proteinaceous inclusions called Lewy bodies. Little is known about why and how the neurodegenerative process begins and progresses, however it has been hypothesized that PD could have a multifactorial etiology, including external factors and genetic predisposition. PD clinical manifestation consists mostly of motor-related symptoms such as bradykinesia, akinesia, muscular rigidity and resting tremors. L-DOPA, the catecholamine precursor, is the gold standard in treating PD, although its effectiveness decreases over time and the prolonged administration is associated to severe side effects such as dyskinesia. Among the alternative therapeutic strategies, neurotrophic factors such as GDNF, either administered as recombinant protein or continuously expressed by adeno-associated virus-mediated gene therapy, demonstrated efficacy with enhanced neuroprotection. Recently, we described a novel long non-coding RNA (lncRNA) that is able to increase the protein synthesis at post-transcriptional level. This led to the development of a set of synthetic antisense RNA as potential enhancers of the translation of endogenous target mRNA. This class of synthetic RNA is called SINEUP, since their activity requires an invSINEB2 element (SINE) to UP-regulate the translation of partially overlapping sense mRNAs. This novel class of genetic modulators could be potentially a powerful tool in neurodegenerative disorders by mediating the overexpression of neuroprotective factors. Here, we described the molecular effects of a novel SINEUP targeting GDNF *in vitro* and in preclinical experimental models of PD.

S50.2
A NEURO-SPECIFIC GENE THERAPY APPROACH TO TREAT COGNITIVE IMPAIRMENT IN DOWN SYNDROME BY RNA INTERFERENCE

Andrea Contestabile
Istituto Italiano di Tecnologia. Department of Neuroscience and Brain Technologies, Italy

Down syndrome (DS) is a genetic disorder caused by the presence of a third copy of chromosome 21. Trisomic mouse models of DS reproduce the main cognitive disabilities of the human syndrome. In particular, the Ts65Dn mouse model of DS shows learning and memory deficits, largely determined by altered GABAergic transmission through chloride-permeable $GABA_A$ receptors ($GABA_AR$). Specifically, we have recently found increased expression of the chloride importer NKCC1 (Na-K-Cl cotransporter) in the brains of both Ts65Dn mice and DS patients. Accordingly, intracellular chloride accumulation in the adult trisomic brain shifts the chloride reversal potential (E_{Cl}) toward more positive values and $GABA_AR$-mediated signaling from inhibitory to excitatory. The identification of NKCC1 as a pivotal molecular target for the rescue of cognitive deficits in DS opens the possibility of a gene therapy approach to treat the disease. Here, to normalize NKCC1 expression and rescue synaptic dysfunctions as well as cognitive deficits in trisomic mice we have developed and characterized a knock-down approach to normalize intracellular chloride accumulation. Reducing the expression of NKCC1 by RNA interference restored $GABA_AR$-mediated inhibition in trisomic neurons *in*

vitro. Most importantly, AAV-mediated neuron-specific NKCC1 knockdown *in vivo* in the hippocampus of adult Ts65Dn mice rescued behavioral performance on different learning and memory tests at levels undistinguishable from those of WT mice. Our findings demonstrate that NKCC1 overexpression drives intracellular chloride accumulation in trisomic neurons, leading to depolarizing GABAAR signaling and behavioral impairments in DS mice. Moreover, our study identifies a new gene therapy target for treatments aimed at rescuing cognitive disabilities in individuals with DS.

S50.3
PRECLINICAL MODELLING OF LENTIVIRAL-MEDIATED CNS GENE TRANSFER IN RODENTS AND NON-HUMAN PRIMATES

Angela Gritti
San Raffaele Telethon Institute for Gene Therapy (SR-Tiget), Division of Regenerative Medicine, Stem Cells and Gene Therapy, IRCCS San Raffaele Scientific Institute - Milano, Italy

In vivo gene therapy (GT) is emerging as a promising strategy to treat brain diseases. Adeno-associated virus (AAV) vectors and lentiviral vectors (LV) are both promising GT vehicles for CNS-directed applications. AAV-based GT trials have initiated to unveil the therapeutic potential of this approach for CNS disorders. More recently, the first clinical trial employing a LV to deliver dopaminergic genes in Parkinson disease (PD) patients has been performed, with promising results in terms of safety and efficacy. The ideal vector for GT should have a few key properties, including low immunogenicity, lack (or low risk) of oncogenicity and pathogenicity, efficient gene transfer, long-term expression of the transgene product, and scalable manufacture for clinical applications. Because of their peculiar biological properties, LV may represent an alternative platform that might be tailored to specific applications, in order to address some of the limitations and complement the reach of AAV vectors that are currently in an advanced stage of clinical testing. Our group has long invested in the development of a LV-mediated CNS-directed GT platform for the treatment of metachromatic leukodystrophy (MLD) and globoid cell leukodystrophy (GLD, or Krabbe disease), two severe neurodegenerative lysosomal storage diseases (LSD) caused by arylsulfatase A (ARSA) and Galactosylceramidase (GALC) deficiency, respectively. Our studies established the LV GT platform as a rapid and effective intervention to provide pervasive supply of therapeutic lysosomal enzymes in CNS tissues of MLD and GLD mice. Importantly, data obtained in mice were recently replicated in large disease animal models. In particular, we demonstrated favorable safety profile and remarkable biodistribution of transgene products upon of LV-mediated GT in normal as well as in GLD affected non-human primates (NHP). Our results support the rationale for the clinical translation of intracerebral LV GT to address CNS pathology in MLD, GLD and other neurodegenerative LSD. Also, they pave the way to the development of combined approaches able to timely address the multi-organ pathology of these severe diseases.

S50.4
RESCUE OF THE PERIPHERAL NERVOUS SYSTEM IMPAIRMENT IN POMPE DISEASE MOUSE MODEL BY LIVER GENE TRANSFER

Giuseppe Ronzitti
Genethon, Evry, France

Pompe disease is a rare disease due to the deficiency of the lysosomal enzyme acid α-glucosidase (GAA) that is involved in glycogen lysosomal degradation. In Pompe disease patients, the accumulation of glycogen causes cardiomegaly with respiratory distress, muscle weakness and feeding difficulties. The only therapeutic option for Pompe disease is Myozime, the recombinant form of GAA. Although very successful in preventing heart impairment, the main limitation of Myozime is its poor biodistribution. It has been demonstrated that Myozime efficiently rescue the glycogen accumulation in the heart and at a lower extent in the diaphragm. Limited effects have been observed in the skeletal muscles with small improvement in the locomotor function. Also biodistribution studies demonstrate that, at the doses used in the clinic, Myozime does not cross the blood-brain barrier being unable to correct the disease in central and peripheral nervous system. Studies conducted in the mouse model of the disease demonstrate that the respiratory impairment is partially dependent on the glycogen accumulation in the peripheral nervous system. Therefore, Pompe disease is a multi-systemic disorder and an appropriate therapy for Pompe disease should achieve a full-body correction of the disease. Several gene therapy approaches have been developed in the past to treat Pompe disease but none of them achieved a full body correction of the disease phenotype. In an effort to develop an efficient gene replacement therapy for Pompe disease we optimized the liver depot strategy, that has been already exploited in different lysosomal storage disorder. Following this strategy, we used an adeno-associated virus (AAV) vector to express GAA in the liver fused with an efficient signal peptide and we achieved an efficient secretion of GAA in the bloodstream. We demonstrated that the high levels of circulating GAA were able to completely correct the respiratory impairment, partially due to a defect in the diaphragm innervation and to the glycogen accumulation

in the spinal cord. This study demonstrates for the first time that the AAV-mediated expression of GAA in the liver can be used to achieve a full-body correction of Pompe disease phenotype.

S51 Room: Ballroom
BRAIN DIRECTED GENE TRANSFER STRATEGIES. WHAT WE LEARNED FROM PRECLINICAL RESEARCH FOR RARE DISEASES

Chairs: Nathalie Leresche, FR and Regis Lambert, FR

S51.1
T CHANNEL IN SYNAPTIC TRANSMISSION AND PLASTICITY

Nathalie Leresche
Sorbonne Universités, UPMC Univ Paris 06, INSERM, CNRS, Neurosciences Paris Seine - Institut de Biologie Paris Seine, Paris, France

The role of T-type calcium channels in synaptic transmission and plasticity is seldom considered, a situation that reflects the lack of suitable T-type channel antagonists that till recently has hampered investigations of the functional roles of these channels. However, with the development of new pharmacological and genetic tools, a clear involvement of T-type channels in synaptic function is starting to emerge. Indeed, the presence of presynaptic T-type channels has been recently reported on some synaptic terminals of cortical, hippocampal and dorsal horn neurons. Moreover, activation of presynaptic T-type channels has been shown to contribute to the asynchronous release of transmitters in some brain areas. Furthermore, T-type channels colocalize with proteins involved in synaptic release as syntaxin 1A in neurons of the nucleus reticularis thalami and are associated with syntaxin-1A and SNAP-25 in chromafin cells. In this presentation, we will show that pre-synaptic T-type channels control the spontaneous release of GABA at the synapses between neurons of the nucleus reticularis thalami and thalamocortical neurons of the rat somatosensory nucleus. Moreover at this inhibitory synapse, T-type channels located post-synaptically on thalamocortical neurons contribute to long-term synaptic plasticity. By combining *in vitro* electrophysiological recordings and calcium imaging, we showed that long-term depression (I-LTD) develops at this inhibitory synapse when it is challenged by a stimulation paradigm that mimics thalamic network activity occurring during sleep slow waves. The mechanism underlying this plasticity presents unique features: a) it is both heterosynaptic and homosynaptic in nature, and b) requires calcium entry selectively through T-type channels located on the thalamocortical neuron, and c) depends on activation of the calcium-activated phosphatase, calcineurin. We propose that during slow wave sleep the tight functional coupling between GABA-A receptors, calcineurin, and T-type channels will elicit LTD of the activated GABAergic synapses when coupled with concomitant activation of the metabotropic glutamate receptors that are located postsynaptically to the cortical afferences. This T-dependent I-LTD may be a key element involved in the reshaping of somatosensory information during deep sleep. Overall, these data highlight that in addition to their well-known roles in shaping thalamic firing according to the vigilance states, T-type channels participate in both synaptic transmission and long-term synaptic modification in the intrathalamic network.

S51.2
SURFACE DYNAMICS OF T-TYPE CHANNELS

Martin Heine
Research Group Molecular Physiology, Leibniz-Institute of Neurobiology, Brenneckestrasse 6, D-39118 Magdeburg, Germany

Calcium channel signaling capacity is highly depend on both timing and location of the channel in the cellular membrane. This has been demonstrated for all types of voltage gated calcium channels and has been particular explored in the presynaptic bouton, where calcium channels trigger the neurotransmitter release. Despite the need of very tight coupling between for example calcium channels and synaptic vesicle, or other calcium dependent channels (BK), the stability of such interaction might be variable due to the stochastic motion of proteins in the cellular membrane. Here we have used single particle tracking in order to gain information about the surface mobility of calcium channels due to the constant thermal induced agitation of transmembrane proteins by their surrounding lipids. Using different experimental systems we discovered that most surface expressed calcium channels are mobile and explore membrane regions that are much larger than the proposed critical distance for their calcium dependent signaling capacity. Particular in the presynaptic membrane, CaV2.1 and CaV2.2 channels are mobile and might dynamically regulate the release probability of transmitter vesicles. In order to prove this hypothesis we created a optogenetic approach, based on the light induced clustering of Cryptochrom to manipulate channel dynamics acutely and compartment specific. We found that transient immobilization increase synaptic calcium signals, alters the initial release probability and lead to a pronounced paired pulse depression of the postsynaptic response. Concerning the dynamics of CaV3.2 channels we used a CaV3.2::pHluorin knock-in mouse and probed the impact of channel immobilisation by the

use of specific antibodies/nanobodies against the extracellular pHluorin tag. Here the resonance properties of cultured DRG-neurons was altered, if CaV3.2 channels where immobilised, confirming the altered calcium signaling properties of clustered calcium channels observed in the presynaptic membrane. Our data let hypothesis that the local stochastic mobility of calcium channels is a necessary "noise" in order to keep individual signaling properties of calcium channels. In the synapses the single vesicle release mode can be altered when decreasing the freedom of channel dynamics and might lead to multivesicular release. Thus such manipulation alter dramatically the signaling properties of individual synapses. For CaV3.2 channels their impact in modulation of membrane excitability is also altered and underline the important of calcium channel dynamics in the neuronal membrane. The molecular mechanism, how for example intracellular scaffold protein interactions contribute to the confinement of calcium channels is currently under investigation.

S51.3
CHASING THE ROLE OF T-TYPE CHANNELS IN SPINAL PAIN CIRCUITRY

Emmanuel Bourinet
Institut de Génomique Fonctionnelle, CNRS UMR5203 Inserm U1091 Univ de Montpellier, 141 Rue de la Cardonille, 34094 Montpellier, France

The T-type calcium channel CaV3.2 emerges as a key regulator of sensory functions, but its expression pattern within primary afferent neurons and its contribution to modality-specific signaling remain obscure. We elucidated this issue using a unique knockin/flox mouse strain where CaV3.2 is replaced by a functional CaV3.2-surface-eclipticGFP fusion. We demonstrated that CaV3.2 is a selective marker of two major low-threshold mechanoreceptors (LTMRs), Aδ- and C-LTMRs, innervating the most abundant skin hair follicles. The presence of CaV3.2 along LTMR-fiber trajectories is consistent with critical roles at multiple sites, setting their strong excitability. Strikingly, the C-LTMR-specific knockout uncovers that CaV3.2 regulates light-touch perception, noxious mechanical cold and chemical sensations, and is essential to build-up debilitating allodynic symptoms of neuropathic pain, a mechanism thought to be entirely A-LTMRs-specific. Collectively ours findings support a fundamental role for CaV3.2 in touch/pain pathophysiology, validating their critical pharmacological relevance to relieve mechanical and cold allodynia. The presentation will emphasize on these data and our recent projects.

S52 Room: Carlson Suite
NOVEL APPROACHES TO BRAIN REPAIR

Chairs: Azza Sellami, TN and Bruno Frenguelli, UK

S52.1
SCORPION VENOM COMPONENTS AS POTENTIAL MODELS FOR DRUG DEVELOPMENT IN NEUROPATHOLOGIES

Benkhalifa Rym
Laboratory of Venoms and Therapeutical Molecules, Institut Pasteur de Tunis, Tunis El Manar University, Tunis, Tunisia

Scorpionism represents a major health problem in several countries. More than 1.2 million scorpion stings are registered globally every year. Neurotoxins are responsible for venom toxicity, even if, paradoxically, scorpion venoms were used in these countries, as a source of therapy in traditional medicine. For example, *Buthus martensii Karsch* scorpion is widely used in Chinese ethnomedicine to treat some neurological diseases such as apoplexy, epilepsy, and cerebral palsy. Since the 1940s, scorpion toxins have stimulated many drug discoveries in natural compounds research field. In fact, with the remarkable growth in the number of characterized scorpion venom components, several drug candidates have been found. Scorpion toxins are classified according to their structure, mode of action, and binding site on different ion channels. They present specificity and high affinity and have been used as pharmacological tools to characterize various receptor proteins involved in normal ion channel functioning, as abnormal channel functioning in cases of diseases. Earlier reports showed that scorpion venoms interact with neurotransmitter receptors, as well, such as dopaminergi and G protein coupled like adrenergic and cholinergic receptors. Among these channel, potassium channels has gained the attention of the scientific community studying the nervous processes. In particular, Kv7 potassium channels play crucial role in physiological process involving the function of heart, brain and auditory organs. Mutations in several Kv7 genes are responsible for inherited human diseases linked to membrane hyperexcitability like cardiac arrhythmia, epilepsy or deafness. The Kv7.4 channel is mainly expressed in sensory hair cells of the inner ear. Mutations in the gene encoding Kv7.4 are known to underlie a loss of channel function, responsible of a form of non-syndromic autosomal dominant deafness DFNA2. Recent studies have also shown the implication of Kv7.4 in the regulation of smooth muscle activity and hypertension. Therefore, identifying natural Kv7.4 channels openers might be of therapeutic use and could allow for better understanding of the pathophysiological mechan-

isms. Recently, our team has optimized an experimental process alternating biochemical and electrophysiological tests to identify specific activator(s) of Kv7.4 channels from the venom of the North African scorpion *Androctonus australis hector* (*Aa*). We reported the purification and functional characterization of AaTXKβ(2-64), a new variant of AaTXKβ, from a non-toxic fraction of Aa venom, which acts as the first peptide activator of Kv7.4 channel and Kv7.3 but not Kv7.2. Another "orphan" potassium channel, Kv3.1, is well represented in the central nervous system and especially in the cerebellum. It is involved in the mechanisms of proliferation, differentiation and neuronal degenerative diseases. Currently, experiences are conducted with the *Androctonus australis hector* scorpion major venom fraction, AahG50. This fraction inhibits IKv3.1 with dose-dependent manner. Other researchers, drawing inspiration from mechanisms that toxins use to enter the CNS, identified ligand-mediated strategies that could be used to improve the brain-specific delivery of engineered nanocarriers, including polymers, lipids, biologically sourced materials, and imaging agents.

S52.2
MECHANISMS INVOLVED IN NEUROPEPTIDES INDUCED NEUROPROTECTION: IMPLICATIONS IN OXIDATIVE STRESS NEURODEGENERATION

Olfa Masmoudi-Kouki

Laboratory of Functional Neurophysiology and Pathology, Research Unit UR/11ES09, Department of Biological Sciences, Faculty of Science of Tunis, University Tunis El Manar, 2092 Tunis, Tunisia

Oxidative stress, associated with various neurodegenerative diseases, induces imbalance in ROS generation, impairs cellular antioxidant defences and finally triggers both neurons and astroglial cell death by apoptosis. Astrocytes specifically synthesize and release endozepines, a family of regulatory peptides, including the octadecaneuropeptide (ODN) that has been implicated in neuronal cell proliferation differentiation. We have investigated the potential protective effects of ODN against oxidative cell damages and apoptosis in in vitro models of neurodegenrescence, using neurotoxic agents peroxide hydrogen (H_2O_2) and 6-hydroxydopamine (6-OHDA), and in an *in vivo* model of Parkinson disease (PD), using 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) to trigger alterations of dopamine neurons. We have first observed that the gliopeptide ODN, at very low concentrations (in the picomolar range), exerts a potent protective effect against oxidative stress-induced cells death in cultured cerebellar granule neurons and astrocytes. While ODN has been initially described as an endogenous ligand for central-type benzodiazepine receptors, our results provide evidences that ODN exerts its neuro- and glio-protective actions by interacting with a metabotropic receptor positively coupled to the AC/AMPc and PLC/IPs/calcium transduction pathways. The anti-apoptotic effect of ODN can be ascribed to stimulation of the PKA/PKC/ERK-kinase transduction pathways, which modulates the Bax/Bcl-2 balance in favor of an antiapoptotic activity leading to an inhibition of H_2O_2 and 6-OHDA-induced caspase-3 through the intrinsic apoptotic mitochondrial pathway, and by increasing glutathione production, which in turn attenuates ROS formation. We have also observed that ODN induced a rapid stimulation of SOD and catalase gene transcription and prevents oxidative stress-induced alteration of antioxidant enzyme expression and activities in cultured neuronal cells. In addition, ODN abolishes stress-provoked oxidative damage of biomolecules i.e. formation of lipid oxidation products, malondialdehydes and conjugated dienes, and protein carbonyl compounds. Interestingly, knockdown of ODN precursor gene expression increased vulnerability of glial cells under moderate stress condition. However, addition of ODN (10^{-10} M) to culture media reversed cell death observed in ODN-deficient astrocytes; suggesting that ODN, could be a sensor of oxidative stress and a glio- and neuro-protective agent which may form part of the brain defense mechanisms against oxidative stress injury. Thus to explore the protective action *in vivo*, we have used MPTP-treated mice as PD model. *In vivo* studies reveal that intra-cerebroventricular (icv) injection of 10 ng/µl ODN, in MPTP-treated mice, prevents the reduction of the number of tyrosine hydroxylase-(TH)-positive cell bodies and fibers in the substantia nigra pars compacta and striatum, respectively. Immunofluorescence imaging, Western blot analysis and Q-PCR studies revealed that ODN totally abolished MPTP-induced decrease of tyrosine hydroxylase (TH) positive cells, mRNA expression and protein levels. Furthermore, ODN knockout (KO) mice exhibited more vulnerability to MPTP than wild-type animals. Thus, ODN deficiency protects nigrostriatal dopaminergic neurons to MPTP-induced cell damage and death. By using Q-RT-PCR we observed that interleukin-6 and Tumor Necrosis Factor-alpha mRNA levels increased in the striata in MPTP-treated wild type mice, effects that were significantly strengthened in MPTP-treated KO ODN mice. Furthermore, the expression of the pro-apoptotic caspase-3 enzyme was found enhanced in both MPTP-intoxicated wild type mice and ODN KO animals. The MPTP-induced neuroinflammation and cell death were markedly reduced by icv injection of 10 ng/µl ODN. These results indicate that endogenous ODN may acts to maintain the integrity of

dopamine neurons during PD neurodegenerescence processes. Collectively, these data suggest that astroglia-secreted endozepines may exert an autocrine/paracrine protective effect against oxidative stress and cell death. Thus, the gliopetide ODN and/or stable agonists of the metabotropic ODN receptor might have a therapeutic potential for treatment of cerebral injuries involving oxidative neurodegeneration.

S52.3
THE PURINE SALVAGE PATHWAY AS A THERAPEUTIC TARGET IN BRAIN INJURY

Bruno Frenguelli
School of Life Sciences, The University of Warwick, Coventry, CV4 7AL, UK

Brain injury results in a profound and prolonged depletion of cellular ATP. This arises from the interruption in nutrient supply via vascular occlusion or damage, impaired mitochondrial function in the aftermath of the insult, and the loss of ATP metabolites from the brain into the bloodstream. ATP depletion thus robs the brain of the ability to restore cellular ionic homeostasis, deploy reparative mechanisms and, in addition, results in a diminution of the reservoir for adenosine, a powerful neuroprotectant, anticonvulsant and vasodilator. Using acutely-prepared hippocampal brain slices as a model for the post-traumatic brain, we were able to restore reduced cellular ATP levels to values recorded *in vivo* via the provision of ribose and adenine - two molecules utilised by the purine salvage pathway to generate AMP, which is then further converted to ATP via the actions of adenylate kinase. This greater reservoir of ATP translated into greater release of adenosine in hippocampal area CA1 in response to: theta-burst stimulation of Schaffer axons; oxygen-glucose deprivation (OGD), and epileptiform activity, with corresponding inhibitory effects on synaptic plasticity, synaptic transmission and seizure activity, respectively. Furthermore, the provision of ribose and adenine ("RibAde") protected cerebellar granule cells when given after 6 hours of OGD. To test whether RibAde may be of value in the intact animal after brain injury, we induced focal cerebral ischemia in adult rats via 1 hour occlusion of the middle cerebral artery. We then infused for 6 hours either saline, RibAde or RibAde plus an IP injection of the xanthine oxidase inhibitor allopurinol ("RibAdeAll"). Seven days after occlusion lesion volume was reduced by 18% in the saline group, by 38% in the RibAde group and by 50% in the RibAdeAll group ($p = 0.065$ treated vs untreated; $n = 6$–8/group). Moreover, RibAde-or RibAdeAll-treated animals made faster neurological recoveries from the period of cerebral ischemia over the 7-day period. Given the existing use in man of ribose, adenine and allopurinol, RibAde-based therapy has the potential to be deployed at the point of injury by attending medics to improve cerebral ATP, increase the reservoir of adenosine and thereby reduce the impact of brain injury in a wide variety of emergency and clinical settings.

S53 Room: Carlson Suite
ION CHANNELS AND DISEASES

Chair: Maria Cristina D'Adamo, MT

S53.1
THYMOSIN A1 RECTIFIES THE CFTR-DEPENDENT MULTIFUNCTIONAL DEFECTS IN CYSTIC FIBROSIS

Mauro Pessia
Physiology and Biochemistry, Department of Experimental Medicine, University of Perugia, Piazzale Gambuli Building D-I Floor 06132 Perugia, Italy; Department of Physiology and Biochemistry, University of Malta, Msida, Malta

Cystic fibrosis (CF) is a multi-organ disease resulting in dysfunction of the brain, lungs, gut and pancreas. CF is caused by mutations in the gene encoding the cystic fibrosis transmembrane conductance regulator (CFTR) that compromise its chloride-channel activity. The most common mutation, p.Phe508del, results in the production of a misfolded CFTR protein, which is prematurely degraded. Because of the inherent complexity of the pathogenic mechanisms which include impaired chloride conductance and persistent inflammation a compound, multidrug approach is required for CF therapy. To date, no individual, small-molecule drug with pleiotropic effects is available.

Here we report on the ability of thymosin alpha 1 (Tα1), a naturally occurring polypeptide, to rectify the multifunctional defect in CF mice as well as in patients with the p.Phe508del mutation. Tα1 displayed several combined properties that favorably oppose CF symptomatology: potentiated both the CFTR and calcium-activated chloride currents, increased CFTR maturation and stability and ameliorated multi-organ inflammation. By virtue of this pleiotropic action, Tα1 may be endowed with therapeutic potential in CF.

S53.2
CALCIUM CHANNELS AND MIGRAINE

Daniela Pietrobon
Department of Biomedical Sciences, University of Padova, Italy

Migraine is a common disabling brain disorder whose key manifestations are recurrent attacks of unilateral

headache and interictal hypersensitivity to sensory stimuli. The molecular and cellular mechanisms of the primary brain dysfunction(s) leading to the onset of a migraine attack and to susceptibility to cortical spreading depression (CSD), the neurophysiological correlate of migraine aura and a likely trigger of the headache mechanisms, remain largely unknown and major open issues in the neurobiology of migraine. Gain-of-function mutations in the neuronal CaV2.1 (P/Q-type) calcium channel and loss-of-function mutations in the glial alpha2 Na/K-ATPase cause a rare subtype of migraine with aura: familial hemiplegic migraine (FHM1 and FHM2, respectively). Knockin (KI) mouse models carrying FHM1 or FHM2 mutations show a lower threshold for CSD induction and a higher velocity of CSD propagation. We have investigated the cortical mechanisms underlying the facilitation of experimental CSD in FHM1 and FHM2 KI mice by studying synaptic transmission at different cortical excitatory and inhibitory synapses and the rate of glutamate and K+ clearance by cortical astrocytes in acute cortical slices. Our findings are consistent with the conclusion that increased activation of NMDA receptors due to enhanced cortical glutamatergic synaptic transmission in FHM1 and to reduced rate of glutamate clearance at cortical excitatory synapses in FHM2 contributes to the facilitation of CSD in FHM KI mice. The data from FHM mouse models support the view of migraine as a disorder of brain excitability characterized by dysregulation of the cortical excitatory-inhibitory E/I balance.

S53.3
A CHANNELOPATHY MUTATION IN THE VOLTAGE-SENSOR DISCLOSES CONTRIBUTIONS OF A CONSERVED PHENYLALANINE TO GATING PROPERTIES OF KV1.1 CHANNELS AND ATAXIA

Lara Macchioni

Section of Physiology and Biochemistry, Department of Experimental Medicine, School of Medicine, University of Perugia, Perugia, Italy

A 2 years old boy presents hypothyroidism, with profound developmental delay, failure to thrive, tonic-clonic seizures, ataxia and hypotonia. Targeted and whole-exome sequencing revealed two heterozygous missense variants: a novel mutation in KCNJ10 gene that encodes for the inwardly-rectifying K+ channel Kir4.1 and another previously characterized mutation in KCNT1 that encodes for the Na+-activated K+ channel known as Slo2.2 or Slack. The objective of this study is to examine the functional consequence of the Kir4.1 mutation, the expression of Kir4.1 in human thyroid and to reveal the role the mutation may play in the presentation of a novel phenotype associated with hypothyroidism.

The clinical and genetic characterization of the proband and his family was performed. The mutant and wild-type KCNJ10 constructs were generated and heterologously expressed in Xenopus laevis oocytes, and whole-cell K+ currents were measured using the two-electrode voltage-clamp technique. The presence of Kir4.1 in the human thyroid was determined with immunohistochemical analysis and whole-cell dialyzed configuration of the patch-clamp technique was used for electrophysiological recordings of Kir currents from cultured thyrocytes. The new KCNJ10 mutation c.652C>T resulted in a p.L218F substitution at a highly conserved residue site. Wild-type KCNJ10 expression resulted in robust Kir currents, while currents from oocytes expressing the mutation were reduced. Kir5.1 subunits display selective heteromultimerization with Kir4.1 constituting channels with unique kinetics. The effect of the mutation on Kir4.1/5.1 channel activity was twofold: a reduction in current amplitudes and an increase in the pH-dependent inhibition. Immunohistochemical analysis revealed high expression of Kir4.1 channels in human thyrocytes. Finally, patch-clamp recordings from thyrocytes revealed a high density of inwardly-rectifying K+ currents.

We report a novel loss-of-function mutation in Kir4.1, which was found in a patient with a severely-disabling phenotype that is distinct from EAST/SeSAME syndrome and KCNT1-associated phenotypes. Our results point to a genetic disorder that should be carefully examined in patients with hypothyroidism and global developmental delay and provide new clues to the physiological function of K+ channels in the thyroid.

S53.4
KATP CHANNELS AND NEONATAL DIABETES

Natascia Vedovato

Department of Physiology, Anatomy & Genetics, University of Oxford Parks Road, Oxford OX1 3PT, UK

Pancreatic ATP-sensitive potassium (KATP) channels regulate β-cell membrane potential, calcium influx, and insulin secretion in response to changes in blood glucose levels. This regulation is mediated via alterations in intracellular adenine nucleotide levels. Adenosine triphosphate (ATP) promotes channel closure by binding to the pore-forming subunits (the inwardly rectifying potassium channel Kir6.2; gene KCNJ11), whereas magnesium nucleotides (such as Mg-ATP, MgADP, etc) stimulate channel opening by interacting with the regulatory subunits (the sulfonylurea receptor SUR1; gene ABCC8). The tight link between β-cell metabolism and insulin secretion is jeopardized by mutations in KATP channel genes, which can result in hypo- or hypersecretion of insulin, as in neonatal

diabetes and congenital hyperinsulinism, respectively. Neonatal diabetes is a rare monogenetic disorder that presents within the first 6 months of life. Approximately 50% of cases are caused by activating mutations in the KCNJ11 or ABCC8 genes. Those mutations impair the ability of the channel to close in response to metabolically generated ATP and thereby prevent glucose-induced insulin secretion. KATP channel mutations are also associated with diabetes that presents in later life. A common polymorphism in KCNJ11 (E23K) confers an enhanced risk of type 2 diabetes, potentially by causing a small reduction in ATP inhibition. However, this has been difficult to prove conclusively. To date, all patients with neonatal diabetes due to mutations in the KCNJ11 gene have been heterozygous for the mutation. We have now identified a homozygous mutation (G324R) causing recessive neonatal diabetes. The ATP sensitivity of homomeric G324R mutant channels was slightly but significantly smaller than wild-type KATP channels (IC_{50}, $\sim$ 820 µM). More interestingly, the difference in ATP sensitivity between homomeric and pseudo-heteromeric G324R channels was strikingly small (IC_{50}, $\sim$ 8 µM); yet, the proband homozygous for the G324R mutation developed neonatal diabetes, whereas his heterozygous parents were unaffected. Our finding that a tiny difference in ATP sensitivity is sufficient to cause neonatal diabetes may help to resolve the conundrum around the E23K polymorphism. Our data predict that an even smaller reduction in ATP sensitivity (i.e. $<$ 8 µM) may all that is needed to predispose to type 2 diabetes in later life. This difference in ATP sensitivity may be hard to resolve experimentally, and thus account for the differences in the functional effects of the E23K variant reported by different labs.

S54 Room: Marie Louise 2 INTEGRATED REGULATION OF STRESS- AND AROUSAL-RELATED BEHAVIOURS BY RELAXIN-3/RXFP3 SYSTEMS

Chairs: Anna Blasiak, PL and Andrew Gundlach, AU

S54.1 PEPTIDERGIC MODULATION OF PAIN: EFFECTS OF RELAXIN-3 ON DESCENDING CONTROLS

João Covita
Bordeaux University, IINS, CNRS UMR 5297, Bordeaux, France; The Florey Institute of Neuroscience and Mental Health, Parkville, Australia

Pain is a complex biological process that plays a vital role in survival, but persistent, intense pain can become a hindrance to normal physiological function and behaviour with a serious negative impact on quality-of-life. In fact, patients with persistent pain conditions routinely develop comorbid symptoms such as anxiety and sleep disorders that worsen pain sensation, creating a positive feedback mechanism between pain and these comorbid conditions. Notably, these comorbid symptoms have been linked to brain areas/circuits that are modulated by the neuropeptide, relaxin-3. Since its discovery in 2001, relaxin-3 has been linked to the control of a range of behaviours in rats and mice including anxiety [1,2], arousal [3,4] and motivated, reward-seeking behaviours [5,6], via activation of its cognate receptor, RXFP3. The involvement of relaxin-3/RXFP3 signalling in these brain functions led us to explore a possible link between central activation of RXFP3 and control of pain sensitivity. Firstly, we assessed the effect of RXFP3 activation/inhibition on the control of mechanical and thermal pain sensitivity in normal and persistent pain conditions. We demonstrated that central intracerebroventricular (icv) administration of the RXFP3 agonist peptide, RXFP3-A2, produced relief of mechanical but not thermal pain sensation in adult mice. Moreover, icv injection of the RXFP3 antagonist peptide, R3(B1-22)R, augmented mechanical and thermal pain sensitivity. These data suggest that relaxin-3 has a tonic effect in maintaining mechanical and thermal pain thresholds, and that there is scope to explore activation of RXFP3 to produce pain relief. Additionally, we sought to identify the neural circuits linked to the observed effects. We used the retrograde tracer, fluorogold, to determine which of the brain areas that receive relaxin-3 inputs are connected to the rostroventral medulla (RVM), a region that acts as a gateway for descending pain control signals. Fluorogold immunoreactivity was detected in neurons in the vicinity of relaxin-3 inputs in the central amygdala, hypothalamus and the bed nucleus of the stria terminalis. These areas have been reported to be related to pain sensation, and impairment of their function has been linked to pain-associated comorbid symptoms.

Together, our data suggest RXFP3 as a potential therapeutic target for producing improved pain management, although further studies targeting the specific mechanisms that underlie such control are warranted.

S54.2 CELLULAR MECHANISMS ASSOCIATED WITH RELAXIN-3'S OREXIGENIC ACTION AND ITS INTERACTIONS WITH AROUSAL AND STRESS SYSTEMS

Anna Blasiak
Department of Neurophysiology and Chronobiology, Jagiellonian University, Krakow, Poland

Optimal food intake and energy metabolism are essential for the survival of individuals and consequently whole species, and therefore evolutionary pressure has led to sophisticated regulatory control of these processes by key motivational neuronal circuits within the hypothalamus, midbrain and brainstem. The maintenance and fine tuning of energy intake/expenditure and optimal body weight is adapted to the environmental conditions and the needs of the organism, via reciprocal interconnections and interactions between different neural networks sensitive to both external and internal factors such as stress, circadian and seasonal variations, food availability and peripheral metabolic hormones and nutrients. However, in modern societies an overabundance of life-related psychological stressors and free access to highly-rewarding, high-caloric foods, combined with the sensitivity of energy homeostasis circuits to external influences, have led to eating disorders. Among these, overweight and obesity are the most common and obesity is a leading preventable cause of death globally. Thus, a better knowledge of the neural control of energy homeostasis is very important therapeutically. Energy homeostasis regulation engages many brain neurotransmitter systems including amino acids, monoamines and peptides. Following the discovery of the neuropeptide, relaxin-3, its first identified physiological effect after central administration, was an increase in food intake in satiated adult rats, with a similar potency to canonical orexinergic peptides such as neuropeptide Y. Relaxin-3, the ancestral member of the relaxin peptide family, is highly expressed in rat, mouse and non-human primate brain. Relaxin-3/GABA neuron populations have been identified in the nucleus incertus (NI), pontine raphe nucleus, periaqueductal grey (PAG) and an area dorsal to the substantia nigra. Evolutionary conservation of relaxin-3 suggests a critical biological function. Relaxin-3 binds with high affinity to the G-protein-coupled receptor, relaxin-family peptide 3 receptor (RXFP3). Importantly, NI relaxin-3 neurons are highly responsive to a range of stressors, which strongly activate relaxin-3 synthesis. Direct sensitivity of relaxin-3 NI neurons to the stress/arousal peptides, CRF and orexin, and the potent orexinergic peptides, orexin, dynorphin and ghrelin, suggest the relaxin-3/RXFP3 signaling system as a candidate for linking stress and stress-sensitive behaviours, including feeding. A possible neural substrate for mediating the orexinergic effect of relaxin-3 is the hypothalamic paraventricular nucleus (PVN) neurons synthetizing the anorexigenic peptide hormones, oxytocin and vasopressin. In rat brain slices, activation of RXFP3 hyperpolarized a majority of magnocellular oxytocin and vasopressin PVN neurons recorded. Patch clamp studies revealed that the inhibitory action of RXFP3 activation on PVN neurons was direct, since it persisted in the presence of GABA and glutamate receptors antagonists and tetrodotoxin. Moreover, *in situ* hybridization histochemistry revealed a strong colocalization of RXFP3 mRNA with oxytocin and vasopressin immunoreactivity in PVN neurons. The effect of RXFP3 activation depends on a calcium conductance, as the presence of cadmium, a calcium channel blocker, antagonized these actions. RXFP3 activated intracellular pathways dependent on Gi/o-proteins, since pertussis toxin, an irreversible inhibitor of $G_{i/o}$-proteins, in the recording pipette during patch clamp experiments, prevented the inhibitory action of RXFP3 activation in the majority of PVN neurons tested. A strong and direct inhibitory influence of relaxin-3/RXFP3 signalling on PVN magnocellular cells, along with an abundance of peri-PVN relaxin-3-immunoreactive nerve fibers, originating from the stress-sensitive neurons of the nucleus incertus, support the hypothesis that relaxin-3/RXFP3 signalling is associated with acute and/or chronic stress and feeding/metabolic-related abnormalities.

S54.3
ROLE OF GABA/PEPTIDERGIC NEURONS IN THE NUCLEUS INCERTUS IN CONTROL OF AROUSAL AND EMOTIONAL COGNITION

Sherie Ma
The Florey Institute of Neuroscience and Mental Health, Parkville, Victoria, Australia

Arousal and vigilance are essential for survival and relevant regulatory neural circuits lie within the brainstem, hypothalamus and forebrain. *Nucleus incertus* (NI) is a distinct region within the pontine periventricular grey, containing a substantial population of large GABAergic neurons with long-range, ascending projections. Efferent and afferent connections implicate the NI in processes of 'behavioural planning, habenular function, hippocampal/cortical activity in attention/memory, and in oculomotor control'. The NI is a site of corticotropin-releasing factor (CRF) action, and forms a neural circuit positioned to modulate arousal and stress responses and the de/synchronization of hippocampal theta rhythm, an oscillatory activity (4–12 Hz) prominent in the electroencephalograph (EEG) during exploration and memory processes. Theta rhythm underlies goal-oriented behaviour and cognition, and is a neurophysiological signature of REM sleep. The NI is also a primary site for neurons expressing the neuropeptide, relaxin-3, which can modulate septohippocampal activity and theta rhythm and is associated with spatial working memory in the rat. However, NI function in awake, behaving animals remains unclear. Therefore, in recent studies, we used a chemicogenetic approach to modulate NI activity in freely-moving rats and assessed the behavioural and physiological con-

sequences. Briefly, in adult male Sprague-Dawley rats, an adeno-associated viral vector was used to transduce NI neurons with excitatory or inhibitory DREADDs (i.e. designer receptors exclusively activated by designer drugs), which are modified human muscarinic receptors (hM3Dq or hM4Di). For behavioural and EEG recordings, DREADDs were activated by the specific designer ligand, clozapine-N-oxide (CNO). hM3Dq activation resulted in long-lasting depolarization of NI neurons with action potentials *in vitro*. Peripheral injection of CNO significantly increased c-Fos immunostaining in the NI and promoted cortical electroencephalograph (EEG) desynchronization. These changes were associated with heightened arousal, and increased locomotor activity in the homecage and a novel environment. NI activation also altered responses in a fear conditioning paradigm, reflected by increased head-scanning, vigilant behaviour during conditioned fear recall. CNO-induced inactivation of hM4Di-expressing NI neurons impaired spatial memory performance, similar to lidocaine blockade of NI. Our findings provide direct evidence that the NI promotes general arousal via a broad behavioural activation circuit and support early hypotheses based on its connectivity, that the NI is a modulator of cognition and attention, and emotional and motivated behaviours.

S54.4
MODULATION OF SEPTOHIPPOCAMPAL FUNCTION BY NUCLEUS INCERTUS AND RELAXIN-3/RXFP3 SIGNALLING

Francisco E. Olucha-Bordonau
Department of Medicine, School of Health Sciences, Universitat Jaume I, Castellón de la Plana, Spain

Hippocampal-dependent cognitive functions are modulated by ascending projections arising from the basal forebrain, hypothalamus and brainstem. One consequence of this modulation is the emergence within the hippocampus of a synchronizing wave of 4–12 Hz frequency known as 'theta rhythm'. This rhythm is highly associated with locomotion, arousal and cognitive processes and an important node for this modulatory rhythm is the *nucleus incertus* (NI) in the brainstem. Neural tract-tracing methods have revealed that the NI projects to all centres involved in subcortical induction/modulation of hippocampal theta rhythm which includes the median raphe, supramammillary nucleus, medial septum and hippocampus. Electrical activation of the NI increased hippocampal theta in urethane anesthetized rats and lesion of the NI abolished the hippocampal theta induced by stimulation of the *nucleus reticularis pontis oralis* (RPO); indicating that RPO induction of theta occurs via the NI. Relaxin-3 is a highly-conserved peptide produced by a population of NI neurons that is present in NI target areas, where it acts via the $G_{i/o}$-protein-coupled receptor, RXFP3. Indeed, several neuronal types in the medial septum have been identified as targets of NI relaxin-3 nerve fibres, which contain 'oval' vesicles and display symmetrical contacts, which are characteristic of inhibitory actions. In addition, the NI receives descending projections from the medial septum, which arise from calretinin-containing neurons. Injection of an RXFP3 agonist into the septum induced an increase of hippocampal theta, while an RXFP3 antagonist injection impaired hippocampal theta induced by RPO stimulation. Electrophysiological recordings reveal that relaxin-3 positive NI neurons fire during the ascending phase of hippocampal theta field potentials in CA1 field. Several recent lines of evidence have indicated a significant role for the NI and relaxin-3/RXFP3 signalling in the control of locomotor activity. Microstimulation of the NI induced an increase in locomotor activity. Excitatory DREADD-induced activation of NI neurons produced cortical electroencephalograph desynchronization, reflecting a heightened arousal, which was associated with a sustained increase in locomotor activity in both the homecage and a novel environment. Furthermore, icv injection of a specific RXFP3 agonist (RXFP3-A2) increased locomotor activity in a T-maze. This treatment was associated with an activation of ERK phosphorylation in cholinergic neurons of the medial septum at 20 and 90 min post-treatment, and disruption of a spontaneous alternation task (a 10 min test at 90 min post-treatment), consistent with an impairment of spatial working memory. This result contrasts with a similar effect observed after an intraseptal blockade of RXFP3, and may reflect differential effects of local vs more broad regional RXFP3 modulation. Together, these findings reveal that the ascending pathway from NI to medial septum plays an important role in the induction/modulation of hippocampal theta rhythm and associated arousal and cognitive mechanisms, with clear evidence that these actions are regulated by relaxin-3 signalling.

S55 Room: Reading Room
COMMON MECHANISMS IN PATHOPHYSIOLOGY OF NEURODEGENERATIVE DISORDERS

Chairs: Ioannis Sotiropoulos, PT and Sheela Vhas, FR

S55.1
NOVEL SYNTHETIC MICRONEUROTROPHINS AS NEUROPROTECTIVE AND NEUROGENIC AGENTS AGAINST NEURODEGENERATION

Ioannis Sotiropoulos
Life and Health Sciences Research Institute (ICVS), Medical School, University of Minho, Portugal; ICVS/3B's - PT Government Associate Laboratory, Braga/Guimarães, Portugal

Synaptic malfunction is a key pathomechanism in both depressive and Alzheimer's disease (AD) pathologies with chronic stress and stress hormones, glucocorticoids (GC), being a risk factor for both disorders. Accumulating evidence has suggested the continuum between depression, impaired cognition and AD raising stress, a well-known sculptor of brain plasticity, as potential connecting factor. As Tau protein and its hyperphosphorylation have been implicated in neuronal/synaptic malfunction in AD brain, we hereby assessed whether Tau plays a critical role in stress-driven depressive pathology and associated cognitive decline. For that purpose, we exposed Tau knock-out (Tau-KO) mice and their wild-type (WT) littermates in chronic unpredictable stress providing behavioral, electrophysiological, ultrastructural, molecular and proteomic analysis. Our recent findings demonstrate, for the first time, that stress- and GC-driven neuronal deficits in wild-type mice are accompanied by synaptic missorting of Tau and enhanced Fyn/GluN2B-driven synaptic signaling assessed by both molecular (WB) and ultrastructural (TEM) analysis (Lopes et al., PNAS 2016; Pinheiro et al., Mol Neurobiol 2016). In contrast, mice lacking Tau (Tau-KO) Tau-KO mice are resilient to chronic stress exhibiting no depressive-like behavior and cognitive impairments while, in contrary to WT, stressed Tau-KO also did not display neuronal/synaptic atrophy, reduction in synaptic plasticity and MRI-based neuronal activity (Lopes at al., PNAS 2016). Furthermore, our quantitative proteomic analysis of synaptosomal fractions, combined with TEM analysis, suggested a prominent role for mitochondria in the regulation of the effects of stress affecting their synaptic localization and function (Lopes et al., 2016 Cerebr Cortex). These findings identify Tau as an essential mediator in the orchestration of cellular cascades underlying dendritic and synaptic atrophy/loss in stress-evoked depressive pathology and associated cognitive deficits adding to our molecular understanding of how stress precipitates brain pathology.

S55.2
STRESSFUL EXPOSURE AND SEX DIFFERENCES IN BRAIN VULNERABILITY TO DEPRESSIVE PATHOLOGY: IMPLICATIONS FOR DEMENTIA

Christina Dalla
Dept. of Pharmacology, Medical School, National and Kapodistrian University of Athens, Greece

Clinical studies support that depression maybe a risk for Alzheimer disease (AD) suggesting common neurobiological basis between both diseases. Interestingly, both disorders exhibit a clear sex-dependent epidemiological profile with women being more prone to these disorders; still the biological mechanisms underlying such sex differences remain unclear. This talk will provide evidence from our team clarifying the sex-dependent vulnerability of different brain areas and circuits to depressive and AD pathologies using different animal models of depression e.g. Forced swim test (FST) or chronic mild stress (CMS) and AD Tg mouse. Our findings from FST and CMS models demonstrate that depressive-like symptomatology is more evident in females than males, accompanied by decreased serotonergic turnover ratio in female, but not male, hippocampus. Interestingly, these sex differences in depressive-like behavior do not depend on the differing corticosterone levels between the male and female animals. Moreover, antidepressant treatment also revealed clear sex differences in molecular and behavioral response suggesting a sexually dimorphic brain response to environmental or pharmacological treatment. In another set of experiments, we have also demonstrated that female hippocampus of AD Tg mice was more vulnerable to chronic stress identifying the differing cellular mechanisms. Trying to understand the differing brain vulnerability, we have recently identified a novel brain area of the hippocampus-prefrontal cortex circuitry whose disruption blocked the establishment of stress-driven depressive pathology. Our findings point to sex-dependent neurobiological and behavioral response of male and female brain to stress, highlighting sexually dimorphic neurochemical and molecular insights of depressive and AD pathologies. Future exploration of these sex-dependent brain differences will help us to improve our mechanistic understanding of sex differences in these disorders.

S55.3
CYCLOPHILIN MALFUNCTION UNDERLIES THE DEVELOPMENT OF DISTINCT LATE-ONSET, NEURODEGENERATIVE MALADIES

Ehud Cohen
Biochemistry and Molecular Biology, the Institute for Medical Research Israel – Canada; (IMRIC), the School of Medicine of the Hebrew University of Jerusalem, Jerusalem 91120, Israel

Neurodegenerative disorders (ND) including Alzheimer's (AD), Prion and Huntington (HD) diseases share two key features; they stem from aberrant protein aggregation (proteotoxicity) and onset late in life. The common temporal emergence pattern of

maladies that exhibit distinct etiologies defines aging as the major risk factor for the development of these disorders and suggests that the aging process exposes the elderly to disease by compromising the activity of mechanisms that prevent their manifestation early in life. Although extensive research established the mechanistic links of aging to proteotoxicity, how aging enables the accumulation of protein aggregates late in life is largely obscure. Here we focus on the effects of aging on early steps of protein folding, searching for similar mutations that lead to the development of different neurodegenerative disease as hints towards the identification of shared folding chaperone recognition sites and found that similar proline substitutions in the motif PXXP in the sequences of the prion protein (PrP) and presenilin1 (PS1) underlie the development of the familial prion disorder GSS and to early-onset AD respectively. The inhibition of folding chaperones of the cyclophilin family by cyclosporin-A (CsA) resulted in the misfolding and deposition of wt PrP and of PS1 in peri-nuclear deposits. While misfolded PrP accumulates in cytosolic aggresomes, aberrantly folded PS1 is deposited in the ER quality Control compartment (ERQC) following CsA treatment. We found that the misfolding of PS1 by CsA has led to the impairment of its proteolytic activity and to impaired mitochondria distribution and function. Investigating the nature of PrP containing aggresomes we discovered that these structures serve as components of an ER protein quality control mechanism. Our discoveries point at cyclophilin B as a novel linker between proteotoxicity, the aging process and neurodegenerative diseases and demonstrate a single failure that underlies the manifestation of distinct disorders.

S55.4
GLUCOCORTICOID SIGNALING IN NEURODEGENERATIVE PATHOLOGY OF PARKINSON'S DISEASE

Sheela Vhas

Laboratory of Gene Regulation and Adaptive Behavior, INSERM U1130, CNRS UMR8246 and Sorbonne, Universités, Paris, France

Parkinson's disease (PD) is a complex multisystem neurodegenerative disease characterized by not only motor symptoms but also behavioral anomalies including depression, anxiety and cognitive deficit. Pathologically PD is characterized by loss of dopaminergic neurons in substantia nigra, presence of Lewy bodies and chronic inflammation. Several studies have shown high circulating cortisol in PD patients; as well we previously reported reduced levels of glucocorticoid receptor (GR) in substantia nigra indicating an involvement of glucocorticoid (GC) signaling in PD pathogenesis. GCs through GR exert widespread adaptive actions impacting behavior, immune functions as well as neuronal survival. However when GC signaling is compromised, for example as a result of chronically elevated GCs levels, absence of or altered GR activity can influence neurodegenerative processes. Thus using mouse models in which GR is inactivated selectively in microglia or astrocytes we have assessed cell-specific actions of GR in experimental Parkinsonism. Our results show an increased loss of substantia nigra dopaminergic neurons with significant microglial activation and increased expression of pro-inflammatory mediators in the absence of GR either in microglia or in astrocytes. Furthermore, microglial GR profoundly affects its functions, which has an impact on neuronal survival. These actions of microglial GR range from regulation of TLR9 activation to motility of microglia. In astrocytes, GR was observed to regulate connexin hemichannel opening thereby affecting inflammatory parameters and dopamine neuronal survival. To gain further insights into GC-GR alterations in PD, we are analyzing GR expression in microglia and in astrocytes of post-mortem PD brain tissue. In conclusion, our combined work in human PD and in mouse models suggests that altered GC signaling modulates PD pathology opening an opportunity for therapeutic intervention.

S56 Room: Marie Louise 1
NEW PERSPECTIVES IN AMYOTROPHIC LATERAL SCLEROSIS: FROM MOLECULAR BASIS TO THERAPEUTIC INTERVENTION

Chair: Giuseppe Pignataro, IT

S56.1
PRECONDITIONING INDUCED BY LOW DOSES OF LBMAA IN SOD1G93A MICE MODULATES THE IONIC TRANSPORTER NCX3 LEADING TO A STATE REFRACTORY TO ALS

Giuseppe Pignataro

Division Pharmacology, Department Neuroscience, School of Medicine, Federico II University of Naples, Naples, Italy

The present study characterized for the first time an animal model of preconditioning in ALS. Preconditioning (PC) is a phenomenon wherein a mild insult induces a cellular and tissue resistance to a later severe injury. Here we demonstrated that, PC, induced by low doses of LBMAA, elicits gene expression changes leading to a state refractory to ALS. First of all we characterized the first preconditioning mouse model of ALS based on subthreshold treatment with the toxin LBMAA and then

we demonstrated that the plasmamembrane exchanger $Na+/Ca^{2+}$, NCX3, represents a target for setting on new strategies in ALS intervention. PC was induced by icv injection of low doses of LBMAA. The effect of PC was evaluated on disease onset, on motor functions and on motoneurons in terms of functional declines and severity of histological damage. LBMAAinduced preconditioning prevented the downregulation of NCX3 in the spinal cord of G93A mice and reduced neuroinflammation. Interestingly, LBMAAinduced preconditioning determined an increase of survival and a better behavioral motor task performance in G93A mice.These studies allowed us to setting on the first model of preconditioning in ALS and to candidate NCX3 as a new target for setting on new ALS therapies. In particular, the expression of NCX3 protein decreased in the amyotrophic lateral sclerosis affected areas during aging.Interestingly, the preconditioning treatment prevented the downregulation of NCX3. Moreover, we found an increase of NCX3 signaling in neuromuscular junctions of Preconditioned G93A compared to Vehicletreated G93A mice. Most importantly, the preconditioning stimulus increased the survival rate and improved the motor performance skills. Mice bearing both G93A and NCX3+/mutation showed a more severe worsening of their motor performances, an earlier onset of disease symptoms and a reduced size of gastrocnemious muscle compared to G93A mice. In conclusion, this study candidates NCX3 as a putative target in ALS intervention. The pharmacological activation or the overexpression of NCX3 could mitigate motor neurons degeneration by handling the deregulation in ionic homeostasis occurring in ALS.

S56.2
THE NUCLEAR PORE COMPLEX IS COMPROMISED IN ALS

Jonathan Cory Grima
Johns Hopkins University, USA

Amyotrophic lateral sclerosis (ALS) is the most common motor neuron disease and Frontotemporal dementia (FTD) is the second most common form of early-onset dementia. Interestingly, both of these devastating neurodegenerative diseases share a common genetic mutation in chromosome 9 open reading frame 72 (C9orf72). An expanded hexanucleotide repeat (HRE) [GGGGCC] in intron 1 of the C9orf72 gene is the most common genetic cause of familial and sporadic ALS and FTD, along with Huntington's disease phenocopies. Until recently, very little was known about the underlying mechanisms by which this expanded repeat causes neurodegeneration until 5 independent labs published 3 papers including one from our own group simultaneously showing that dysfunction in Nucleocytoplasmic Transport (NCT) may be a fundamental pathway for C9orf72 ALS-FTD pathogenesis. NCT, the trafficking of protein and RNA between the nucleus and cytoplasm, is critical for signal transduction and is especially arduous for neurons due to their highly polarized biology. Efficient regulation of this process is mediated by the Nuclear Pore Complex (NPC), an extraordinary molecular machine that serves as the main gateway to the nucleus. In order for any cell to function properly, it is imperative that RNA and protein be efficiently and selectively exchanged between the nucleus and the cytoplasm. This critical task is achieved by the $\sim$ 2000 NPCs that span the entire nuclear envelope. Each NPC consists of multiple copies of 30 different proteins called Nucleoporins (NUPs) that differ in anatomical location, function, domain, post-translational modification and residence time. Mutations in various NUPs result in tissue-specific diseases. Interestingly, some of the longest-lived proteins in the mammalian brain are specific NUPs and may represent the "weakest link" in the aging proteome. Finally, 1/3 of all NUPs contain multiple repeats of hydrophobic phenylalanine-glycine (FG) and these FG-NUPs are found along the entire transport route of the NPC. FG-NUPs create an entropic barrier to diffusion through the NPC, are highly dynamic, have short residence times, interact directly with transport receptors, control nucleocytoplasmic transport, and determine the pore permeability limit. We now present data that the NPC is not only compromised in models of C9orf72 but also sALS, suggesting that NPC dysfunction may be a common insult and pathogenic mechanism in the majority of ALS. More specifically, we have surveyed the majority of NUPs in transgenic and BAC C9orf72 mice, iPS neurons, and human postmortem brain tissue using IF, IHC, super resolution imaging, western blot, shRNA, and proteomic analysis. We have identified a unique set of NUPs and transport machinery with critical and disease relevant functions that are consistently affected across not only models of C9orf72 but also sALS, with the majority of these being components of nuclear import machinery that have FG-repeats. Knockdown of FG-NUPs disrupts the ran gradient responsible for fueling nucleocytoplasmic transport and also causes cytoplasmic mislocalization of pTDP43 and critical non-FG-NUPs. General inhibition of nuclear import causes cytoplasmic mislocalization of FG-NUPs and pTDP43 as well as stress granule formation. Finally, all these deficits can be rescued when treating with a potent small molecule inhibitor of nuclear export. This data suggests that NPC dysfunction may be a common insult in the majority of ALS and that disruption of FG-containing NUPs/transport receptors involved in nuclear import may be the first "dominoes" to fall in the disease cascade. Recently, 5 independent labs published 3 papers including one from our own group simultaneously showing that defects in Nucleocytoplasmic Transport (NCT)

may underlie C9orf72-mediated ALS and FTD, 2 devastating neurodegenerative diseases. NCT is mediated by the Nuclear Pore Complex (NPC), the main gateway to the nucleus. We now show that the NPC is compromised not only in C9orf72-ALS but also in sporadic ALS (sALS), which is responsible for 90% of cases. More specifically, we show that a specific family of proteins that make up the NPC is particularly affected. This suggests that NCT dysfunction mediated by defects in the NPC may be a critical global mechanism of ALS and neurodegeneration.

S56.3
TARGETING CYTOSOLIC PHOSPHOLIPASE A2 IN THE SPINAL CORD DELAY THE DEVELOPMENT OF ALS

Rachel Levy
Dept. of Clinical Biochemistry, Faculty of Health Sciences Ben-Gurion University of the Negev and Soroka Medical University Center, Beer Sheva 84105, Israel

Amyotrophic Lateral Sclerosis (ALS) is a fatal multifactorial neurodegenerative disease characterized by selective death of motor neurons in the cortex, brainstem and spinal cord. Cytosolic phospholipase A_2 alpha ($cPLA_2\alpha$) upregulation and activation in the spinal cord of patients with sporadic ALS and in the spinal cord of human mutant SOD1G93A (hmSOD1) transgenic mice was recently reported. To determine the role of $cPLA_2\alpha$ upregulation in the brain and spinal cord in the development of the disease symptoms, its elevated expression was reduced by brain infusion of a specific antisense oligonucleotide against $cPLA_2\alpha$ (AS). Reduction of the elevated expression of $cPLA_2\alpha$ in the spinal cord of hmSOD1 mice by brain infusion of an AS at week 15 (shortly before the appearance of the disease symptoms) for a duration of 6 weeks, delayed the loss of motor neuron function in comparison with hmSOD1 mice and with sense brain infused hmSOD1 mice. To characterize the effect of $cPLA_2\alpha$ upregulation on different processes taking place at the appearance of the disease symptoms, mice were brain infused with AS or with sense at week 15 for 3–4 weeks. The AS treatment that reduced $cPLA_2\alpha$ upregulation in the spinal cord of AS treated hmSOD1 mice (as analyzed at week 18–19), prevented the reduction in the number of the neurons (detected by NeuN), inhibited astrocyte activation (detected by GFAP) and microglia activation (detected by Iba-1 and by CD40). In addition, AS treatment blunted the upregulation of the proinflammatory enzymes Inducible nitric oxide synthase (iNOS) and Cyclooxygenase-2 (COX-2) detected in hmSOD1 mice hmSOD1 mice. The regulatory role of cPLA2α on microglia activation was studied in primary mouse microglia cultures, analyzing CD40 expression. $cPLA_2\alpha$ was shown to regulatesCD40 protein induction in microglia by either LPS or IFNɣ and this regulation is mediated *via* activation of NOX2-NADPH oxidase and NFkB. $cPLA_2\alpha$ is located in the early event induced by LPS mediated by both TRIF and MyD88 pathways. While, under IFNɣ stimulation, $cPLA_2\alpha$ is activated at a later time (4 h) by the autocrine effect of TNFα. We found that the elevation of $cPLA_2\alpha$ protein expression in the spinal cord was first detected at 6 weeks old hmSOD1 mice, and remained elevated during their whole lifespan, long before the development of the disease. The interaction between mutant SOD1 and $cPLA_2\alpha$ in neurons in the spinal cord of hmSOD1 mice is demonstrated, suggesting the expression of the misfolded SOD1 induces the elevation of $cPLA_2\alpha$. In conclusions, since specific reduction of $cPLA_2\alpha$ in the brain stem and spinal cord $cPLA_2\alpha$ significantly attenuated the development of the disease, $cPLA_2\alpha$ may offer an efficient target for treatment of ALS.

S57 Room: Marie Louise 2
ION CHANNEL: A KEY FACTOR IN NEURONAL DYSFUNCTION

Chairs: Rami Yaka, IL, Alex Binshtok, IL and Avi Priel, IL

S57.1
THE ROLE OF M-CURRENT IN NEUROINFLAMMATION

Alexander M. Binshtok
Department of Medical Neurobiology; Institute for Medical Research Israel-Canada, The Hebrew University-Hadassah School of Medicine, Jerusalem; The Edmond and Lily Safra Center for Brain Sciences, The Hebrew University of Jerusalem, Israel

Pain and emotion has long been considered in a close relationship. Anxiety, as a negative emotion is a consequence of chronic pain but might have a causal role in pain worsening as well. Choleycstokinin (CCK) is an anxiety-inducing peptide when it is released into the central nucleus of the amygdala (CeA). It is also known to play a role in pain descending facilitation. Thus CCK is at a crossroad between pain and emotion. In this study, we aimed at investigating the effect of intra-CeA CCK on pain thresholds and nociceptive integration into the spinal cord, in a rat model of inflammatory pain induced by complete Freund's adjuvant (CFA). We showed that intra-CeA infusion of CCK induced both an anti-allodynic effect on awake animals and a reduction of nociceptive activity in spinal dorsal horn neurons, only in animals submitted to inflammatory pain. These analgesic effects are accompanied by CCK- induced increase of CeA neuron excitability as recorded in slices

from inflammatory rats. Such changes in CCK-induced modulation of CeA neuron activity are supported by plasticity of the CCKergic system in the CeA. CCK1 but not CCK2 receptor mRNAs are specifically down-regulated in inflamed rats while CCK immuno-reactivity is increased. We hypothesized that intra-CeA CCK triggers the activation of pain descending control through the midbrain periaqueductal grey (PAG). Thus we assessed the effect of blocking PAG activity on CCK antinociceptive effect. The results showed that after infusing lidocaine into PAG, CCK infusion into CeA had no more effect on spinal nociceptive activity. These results were supported by investigating the anatomical relationships between CCK fibers and retrogradely traced efferent neurons projecting from CeA to PAG. This showed a high level of appositions between CCK terminals and retrogradely traced neurons which were observed by confocal microscopy. All these results showed for the first time (i) an antinociceptive effect of CCK, (ii) changes of intra-CeA CCK functions in inflammatory pain condition and (iii) potential effect of intra-CeA release of CCK in activation of a PAG-dependant descending inhibitory control that reduces the excitability of nociceptive spinal neurons and hence ultimately alleviates pain behaviour.

S57.2
HCN-LINKED DISEASES IN HEART AND BRAIN

Dario Di Francesco
Department of Neurophysiology, Foundation Neurological Institute C. Besta, Milano, Italy; Department of Neurology, San Gerardo Hospital and Laboratory of Neurobiology, Milan Center for Neuroscience, University of Milano–Bicocca, Monza, Italy.University of Milano, Department of Biosciences, The PaceLab, via Celoria 26, 20133 Milano, Italy

HCN channels are widely expressed in cardiac cells and in neurons of the central and peripheral nervous systems, where they serve several important functions. These include pacemaker activity generation and control in cardiac cells, and cellular excitability, generation and modulation of rhythmic activity, dendritic integration, transmission of synaptic potentials and plasticity phenomena in neurons.

As an obvious consequence of their roles, functionally defective HCN channels are natural candidates in the search for inheritable HCN-linked diseases in the heart and nervous system. Much evidence has indeed been accumulated in the last several years indicating that specific HCN4 channel mutations, for example, are associated with sinus arrhythmias, most frequently bradycardia (linked to loss-of-function mutations) but also Inappropriate Sinus Tachycardia (linked to gain-of-function mutations) and other types of more complex arrhythmias. Interestingly, also structural cardiac diseases have been shown to be associated with HCN4 mutations. Evidence has also been collected concerning neuronal HCN-channelopathies. It is now well established that inheritable forms of epilepsy in human patients are linked to HCN1/HCN2 genetic mutations which cause changes in the Ih/If current and increased neuronal excitability. Recent evidence has also emerged showing the involvement of HCN channels in neuropathic and inflammatory pain transmission in DRG neurons, as well as a potential role in the pathogenesis of Parkinson's disease. Some very recent data illustrate the potential diversity of HCN-link pathologies, such as for example the suggestion that Ih channelopathy is involved in autism.

The talk will address early and recent evidence linking HCN dysfunctional mutations and diseases in heart and brain.

S57.3
TIGHTLY REGULATED TRPV1 ACTIVATION BY GQ/GPCR

Avi Priel
The Institute for Drug Research, School of Pharmacy, Faculty of Medicine, The Hebrew University of Jerusalem, Israel

The pain pathway plays a protective role as a senor of tissue damage. Its activation by an array of inflammatory mediators that are released at the site of injury and/or inflammation elicits firing of specialized afferent neurons known as nociceptors. Nociceptors sense the large array of these noxious stimuli through specific receptors such as the 'heat and capsaicin receptor' TRPV1. This pivotal pain receptor channel response to both exogenous (such as plant and animal toxins) and endogenous stimuli to evoke neuronal activation. Endogenously, TRPV1 is activate by a group of molecules known as endo-vanilloids (such as 12-HETE), that are synthesized 'on demand' during injury and/or inflammation, downstream of cellular events as GPCR activation by different inflammatory mediators (such as histamine). The distinct pattern of endo- and exo-vanilloids evoked neuronal activation implicates these stimuli activate TRPV1 through a different mechanism. However, while the activation mechanisms of TRPV1 by exogenous noxious stimuli is vastly investigated, our understanding of the endo-vanilloids evoked response lags behind. We show that endo-vanilloids evoke low-to-moderate TRPV1 activation in both neurons and heterologous systems, in contrast to the robust activation evoked by exo-vanilloids. Moreover, we show that endo-vanilloids -evoked TRPV1 activation is dependent on receptor phosphorylation by PKC. Thus, TRPV1 activation downstream of GPCRs required the activity of multiple signaling cascades, allowing a tight regulation

of this receptor activation and subsequently the pain response.

S57.4
EXCITING INHIBITORY CONTROL OF VTA DOPAMINE NEURONS BY COCAINE

Rami Yaka
The Institute of Drug Research (IDR) School of Pharmacy Faculty of Medicine Hebrew University of Jerusalem, Israel

Drugs of abuse act to increase DA levels above normal level in the reward system. Elevated DA levels cause a feeling of euphoria and well-being; therefore, drug use is reinforced regardless of its many adverse consequences and can eventually result in addiction. Since the VTA is the major source for DA in the reward system and because initiation of drug addiction has demonstrated to occur in the VTA, its regulation is of great importance. The VTA receives excitatory and inhibitory inputs, both of which are modulated by acute and chronic exposure to drugs of abuse. As the excitatory inputs to the VTA are well studied, in the current study we focused on the GABAergic inhibitory inputs. Using rat midbrain slices we have previously shown that acute administration of DA or DA-increasing drugs inhibited $GABA_A$ receptor-mediated inhibitory post-pynaptic currents (IPSCs) in VTA DA neurons. Further, we found that DA-induced inhibition involved activation of DA D2-like receptors or $GABA_B$ receptors and has presynaptic locus determined by measuring paired-pulse ratio and miniatures-IPSCs. However, the use of electrical afferent stimulation in those studies makes the source of the GABAergic inputs to be unknown. We hypothesized that the degree of DA-induced inhibition of $GABA_A$-mediated IPSCs in the VTA will differ in an afferent specific manner. Therefore, optogenetics tools were used in this study to isolate between the different sources of inhibitory inputs innervating the VTA, emerging from the rostromedial tegmental area (RMTg), the lateral habenula (LHb) and the nucleus accumbens (NAc). We found that VTA DA neurons from rats injected with ChR2 in the RMTg presented strikingly greater DA-induced inhibition of light-evoked IPSCs than those recorded in response to afferent electrical stimulation (22% and 76% IPSCs inhibition in electric stimulation or optic stimulation of the RMTg, respectively). Likewise, we found that light evoked NAc-VTA GABAergic inputs was reduced dramatically by dopamine compared with electrical stimulation of afferent fibers. Together these results strongly suggest that region specificity plays a major role in determining the degree of DA-induced inhibition of IPSCs in VTA-DA neurons, eventually controlling their activity. Currently, we are assessing the inhibitory effect of DA application on IPSCs emerging from the LHb and NAc. Understanding the means by which DA modulates GABAergic inputs to the VTA exerted from different afferent pathways will expand our knowledge of how drugs of abuse act in the brain and become addictive.

S58 Room: Ballroom
BRAIN REPAIR AND REGENERATION

Chairs: Afsaneh Gaillard, FR and Fatiha Nothias, FR

S58.1
THE NEURONAL AND ACTIN COMMITMENT: WHY DO AXONS NEED RINGS?

Mónica Sousa
Nerve Regeneration group, Instituto Biologia Molecular e Celular- IBMC and Instituto de Investigação e Inovação em Saúde- I3S, University of Porto, Portugal

Although actin is well recognized as a key player in axon growth, how different actin-binding proteins control its dynamics is still not fully understood. Using the conditioning lesion, a model in which the axon regeneration capacity of spinal dorsal column axons is increased following a priming lesion to the sciatic nerve, we determined that profilin-1 (Pfn1) is increased in regenerating axons whereas the inactive form of the protein is significantly decreased. Profilins provide the pool of competent ATP-actin monomers that can be added to free filamentous actin ends to support their polymerization and growth. Our data shows that *in vitro*, the acute knockdown of Pfn1 severely impairs axon formation and growth. Interestingly, ablation of Pfn1 does not only reduce actin dynamics but it also significantly decreases microtubule growth speed. *In vivo*, mice with an inducible neuronal deletion of Pfn1 have decreased axon regeneration of both peripheral and central DRG axons, further supporting the key role of Pfn1 for optimal axon (re)growth. In vitro, overexpression of constitutively active Pfn1 (S137A) strongly enhanced actin and MT dynamics, and neurite outgrowth. *In vivo* AAV-mediated delivery of constitutively active Pfn1 is currently being conducted. In summary, our work shows that Pfn1 is a determinant of axon regeneration capacity acting as a key regulator of both actin and MT dynamics.

S58.2
UNRAVELLING THE MECHANISMS CONTROLLING NEUROGENIC AND OLIGODENDROGLIOGENIC LINEAGES IN THE ADULT SUBEPENDYMAL ZONE

Felipe Ortega
Biochemistry and Molecular Biology Department IV, Faculty of Veterinary medicine, Complutense University, Madrid, Spain; Institute of Neurochemistry (IUIN), Madrid, Spain; Health Research Institute of the Hospital Clinico San Carlos (IdISSC), Spain

The adult mouse subependymal zone (SEZ) harbors neural stem cells (aNSCs) that give rise to neuronal and oligodendroglial progeny throughout the entire murine life. However, despite the extensive research performed, fundamental questions regarding the cell biology of aNSCs remain to be uncovered. For instance, it is crucial to elucidate whether a single aNSC is capable of differentiating into all the different cell types within their lineage or these distinct progenies constitute entirely separate lineages. Similarly, the cell cycle length, the time and mode of division (symmetric versus asymmetric) that these cells undergo are interesting questions under current investigation. Continuous live imaging and single cell tracking constitutes an excellent tool in order to provide answers to the above essential questions. We have employed an alternative method of aNSCs culture preparation, in which cells isolated from the adult SEZ are kept in absence of growth factors, with the consequence that they maintain their intrinsic neurogenic or oligodendrogliogenic nature, and allows for continuous live imaging by time-lapse videomicroscopy. By using this novel culture preparation, we were able to track single aNSCs and their progeny, characterizing their behavior and their defining hallmarks. Moreover, we have identified different factors that actively regulate the lineage progression of neurogenic or oligodendrogliogenic NSCs, as Wnt proteins EGF/FGF mitogen factors or members of the extracellular matrix. This leads to an improved knowledge regarding crucial aspects of adult NSCs cell biology, opening new perspectives in the future design of therapeutic strategies for brain repair.

S58.3
STEM CELL THERAPY FOR BRAIN DISORDERS

Afsaneh Gaillard
Experimental and Clinical Neurosciences Laboratory, Cellular Therapies in Brain Diseases group, INSERM U1084, Poitiers, F-86022, France; University of Poitiers, Poitiers, F-86022, France

Injury to the human central nervous system (CNS) is devastating due to the poor ability of CNS to self-repair. Neural transplantation has been assessed as a potential approach to restore brain function by replacing lost neurons with healthy new ones. We previously found that grafted foetal cortical neurons could effectively re-establish specific patterns of projections and synapses following adult cortical lesions indicating that cellular repair of cortical and cortical output circuitry is possible. While these studies open the possibility of cell transplantation for cortical repair, the limited accessibility of human foetal cortical tissue constitutes a serious limitation to consider such approaches in a clinical setting. Cortical neurons derived from stem cells offer great potential for cell replacement therapy given their greater accessibility and standard use.

We have found that cortical neurons derived pluripotent stem cells transplanted into cortex of adulte mouse, established specific pattern of projections and synapses corresponding to those of endogenous cortical neurons. These findings demonstrate that transplantation of pluripotent stem cell derived neurons of appropriate cortical areal identity can contribute to the reconstruction of an adult damaged cortical circuit.

S58.4
REGENERATIVE BIOMATERIAL MATRICES FOR TRAUMATIC SPINAL CORD REPAIR

Nothias Fatiha
CNRS UMR-8246, INSERM U1130, UPMC, Neuroscience Paris Seine, NPS-IBPS, Team-Axon Regeneration and Growth-, Université Pierre et Marie Curie, 75005 Paris, France; IMP-ICE/UMR CNRS 5223, Université Claude Bernard Lyon 1 (UCBL) – Villeurbanne, France

The ongoing search for novel, efficient therapeutic strategies for treatment of spinal cord injury (SCI) should greatly profit from the recent progress in the production of innovative biomaterials that when implanted into the lesion site, will function both as extracellular matrix substitute, and as bioactive support structure.

Accordingly and as first step, we developed a therapeutic strategy based on the use of chitosan polymer, that exhibits ideal characteristics for tissue engineering. Biological evaluation of diverse formulations (varying in physical and chemicals features) allowed determining the formulations best suited to integrate into spinal cord tissue. Our experimental paradigm is a thoracic dorsal hemisection in adult female rat, with or without implantation of polymer directly after the lesion. Indeed, implantation of the selected chitosan hydrogel formulation induces (i) strong reduction of the astrocytic reaction, (ii) functional vascularization within the implant, (iii) modulated inflammatory response (iv), and most remarkably, growth of a very high number of axons through the implant, evidence for the material per se being extremely favorable for axon regrowth. Finally, these structural remodeling is associated with an improvement of the partial locomotor recovery. Because it effectively induces neural tissue repair, the chitosan biomaterial may be a promising new approach to treat

SCI.

S58.5
LIFE FACTORS SHAPE ADULT NEUROGENESIS AND HIPPOCAMPAL DEPENDENT MEMORY IN ANIMAL MODELS OF ALZHEIMER'S DISEASE

Jorge Valero Gómez-Lobo
Achucarro Basque Center for Neuroscience and Ikerbasque Basque Foundation for Science, Bizkaia, Spain

Alzheimer's disease(AD) is a neurodegenerative disease characterized by the accumulation of beta-amyloid peptide in the brain, neuronal loss, and dementia. Interestingly, some individuals with high levels of beta-amyloid peptide in their brains do not show clinical symptoms of dementia. These "AD-resistant" individuals are speculated to have a larger "brain cognitive reserve", possibly because of a variety of lifestyle related factors. The term "brain cognitive reserve" refers to the capacity of the brain to maintain normal functioning while confronting injury, disease, or aging. We have taken advantage of our experience analyzing one of the components of the cognitive reserve, hippocampal adult neurogenesis, to evaluate the state of the cognitive reserve. Thus, we have checked the effects of some life factors in adult neurogenesis and memory function in animal models of AD. We analyzed the effect of environmental enrichment (EE), a known positive regulator of the cognitive reserve, and an acute inflammatory stimulus (1 mg/kg lipopolysaccharide, LPS) in hippocampal adult neurogenesis, and memory function of two different animal models of AD (APPswe,ind and 3xTg-AD). We observed a drastic reduction in the production of new neurons in the hippocampus of both mouse models of AD. EE increased the number of newly-generated neurons in the hippocampus of control mice, and rescued memory function and number of new neurons in APPswe,ind mice. On the contrary, LPS administration reduced the production of new-neurons only in the hippocampus of control mice but not in 3xTg-AD mice. Interestingly, LPS impaired memory in 3xTg-AD mice but also in control mice. Our data indicate that the effect of life factors (cognitive stimulation or systemic inflammation) in memory function is prominent in animal models of AD, which show a deteriorated cognitive reserve as indicated by the reduction in adult neurogenesis. Therefore, we propose that life factors are determinant for the functional manifestation of AD.

S59 Room: Clermont Suite
BRAIN IMAGING, HEALTHY AGING, SILENT AND APPARENT NEURODEGENERATIVE DISEASES

Chairs: Demian Battaglia, FR, Driss Boussaoud, FR and Saïd Boujraf, MA

S59.1
COGNITIVE AND EMOTIONAL ALTERATION IN PARKINSON'S DISEASE, BEHAVIORAL AND CELLULAR INSIGHTS INTO NICOTINE AND STRESS INTERPLAY

Boujraf Saïd
Clinical Neuroscience Laboratory, Faculty of Medicine and Pharmacy, Univ. of Fez, Morocco; Depart. of Biophysics; Clinical MRI Methods, Faculty of Medicine and Pharmacy, Univ. of Fez, Morocco

Parkinson's disease (PD), which results from loss of midbrain dopamine neurons, has long been thought to affect mostly motor behavior. However, it is now know well established that cognitive and emotional functions can be severely impaired in patients suffering from PD. This is not surprising given that the dopamine system is at the convergence of limbic (emotional), cognitive and motor systems brain networks. However, we know little on how emotional states impact on motor behavior in PD. In this study, we sought to investigate the functional networks activated by positive, negative and neutral emotions in PD patients using BOLD-fMRI. Fifteen patients were recruited for this study. They were selected on the basis of absence of any additional comorbidity. All patients were tested on a visuomotor learning task typical for activation the fronto-basal ganglia system, and underwent BOLD-fMRI and anatomical MRI using both motor and emotional paradigms. The fMRI data was processed using SPM12 package. As expected based on previous studies (Hadj-Bouziane et al., 2013), learning abilities were impaired in PD patients compared to age-matched control subjects. In parallel, analysis of fMRI data revealed significant changes of brain activation at both cortical and subcortical levels. These changes in brain activations paralleled the variations in emotional and motor performance. These preliminary results demonstrate that altered cognitive and emotional functions lead to learning deficits independently from motor impairments. This talk will address the potential mechanisms through which emotions may affect learning, and discuss future directions in rehabilitation of PD patients.

S59.2
COGNITIVE AND EMOTIONAL ALTERATION IN PARKINSON'S DISEASE

Benzagmout Mohammed
Clinical Neuroscience Laboratory, Faculty of Medicine

and Pharmacy, Univ. of Fez, Morocco; Endocrinology and Diabetology Department, University hospital of Fez, Morocco

Parkinson's disease (PD), which results from loss of midbrain dopamine neurons, has long been thought to affect mostly motor behavior. However, it is now know well established that cognitive and emotional functions can be severely impaired in patients suffering from PD. This is not surprising given that the dopamine system is at the convergence of limbic (emotional), cognitive and motor systems brain networks. However, we know little on how emotional states impact on motor behavior in PD. In this study, we sought to investigate the functional networks activated by positive, negative and neutral emotions in PD patients using BOLD-fMRI. Fifteen patients were recruited for this study. They were selected on the basis of absence of any additional comorbidity. All patients were tested on a visuomotor learning task typical for activation the fronto-basal ganglia system, and underwent BOLD-fMRI and anatomical MRI using both motor and emotional paradigms. The fMRI data was processed using SPM12 package. As expected based on previous studies (Hadj-Bouziane et al., 2013), learning abilities were impaired in PD patients compared to age-matched control subjects. In parallel, analysis of fMRI data revealed significant changes of brain activation at both cortical and subcortical levels. These changes in brain activations paralleled the variations in emotional and motor performance. These preliminary results demonstrate that altered cognitive and emotional functions lead to learning deficits independently from motor impairments. This talk will address the potential mechanisms through which emotions may affect learning, and discuss future directions in rehabilitation of PD patients.

S59.3
COGNITIVE AND EMOTIONAL IMPAIRMENT IN TYPE 2 DIABETES

Driss Boussaoud
Clinical Neuroscience Lab., Faculty of Medicine and Pharmacy, University of Fez, Morocco

Type 2 diabetes (T2D) has been expanding drastically over the last 3 decades and has become a major health crisis worldwide. As T2D patients live longer, brain alterations and cognitive and emotional impairments impact directly their quality of life (McCrimmon et al. 2012). Recent studies demonstrate that brain complications in adults with T2D are associated with cognitive and emotional impairments, but little is known about the relationship between structural alterations and behavioral manifestations. This study has combined BOLD-fMRI and behavioral measures in T2D patients in order to investigate the correlation between cognitive and emotional impairments and structural and functional changes in the cerebral cortex. Twelve patients volunteered in this study. They were diagnosed for T2D at least six months earlier, and had no additional comorbidity. All patients, and age-matched controls, were tested on a visuomotor associative task, and underwent BOLD-fMRI and anatomical MRI using a motor and emotional paradigms. The fMRI data was processed using SPM12 package. The present results are based on preliminary and analysis of behavioral and BOLD-fMRI data of 12 patients and 12 age-matched controls. Analysis of behavioral data showed a decline of learning abilities in T2D patients compared to controls. In addition, T2D patients appeared to display a altered motor and emotional state. Analysis of fMRI data revealed significant changes of activation within a large cortical network including motor cortex, prefrontal cortex and parietal cortex, and that emotional responses and BOLD signal are inversely correlated. These preliminary findings demonstrate that the brain of T2D patients undergoes cognitive and emotional alterations that parallel silent structural degenerative phenomena. Although the causal mechanisms are still to be investigated, the fact that functional impairments can be detected in emotional, cognitive and motor domains calls for the development of preventive measures using neuropsychological tools in T2D patients, and even in pre-diabetic people.

S60 Room: Reading Room
STRESS RESPONDING IN MAMMALS: INTEGRATING CELLULAR SIGNALING, NEURONAL CIRCUITRY, AND BEHAVIOURAL OUTPUTS

Chairs: Youssef Anouar, FR and Lee E. Eiden, USA

S60.1
STRESS-MEDIATED REMODELING OF GAP JUNCTIONAL COMMUNICATION IN THE ADRENAL MEDULLARY TISSUE

Nathalie Guerineau
Institute of Functional Genomics, CNRS UMR5203, INSERM U1191, University Montpellier, France

In mammals, catecholamine secretion from adrenal chromaffin cells represents an ubiquitous mechanism helping the organism to cope with stressful situations. Once delivered into the blood circulation, epinephrine and norepinephrine exert multiple actions, in particular on the cardiovascular system, leading to appropriate adjustments of blood pressure and cardiac rhythm, and on the energy metabolism, enabling the organism to cope with a threat for its survival. While an instantaneous

secretion of catecholamines is beneficial to face an acute stress episode, repeated or prolonged stressful situations resulting in sustained blood catecholamine increases are detrimental for the organism and can initiate many diseases.

Catecholamine secretion from adrenal chromaffin cells relies on both a neurogenic command arising from the splanchnic nerve terminals synapsing onto chromaffin cells and a local gap junction-mediated intercellular communication between chromaffin cells (1). This symposium presentation will be focused on the remodeling of the gap junctional coupling occurring in response to a physiological stress (5 day-cold exposure, male Wistar rats) or a stress-related pathology (arterial hypertension using the animal model of spontaneously hypertensive rats (SHRs)). In acute adrenal slices of cold stressed rats, the gap junction-dependent communication between chromaffin cells is enhanced. This correlates with the appearance of a robust electrical coupling, allowing action potentials to propagate between coupled cells. Accordingly, synchronized spontaneous and nicotine-evoked [Ca^{2+}] rises between chromaffin cells are more frequently observed in stressed animals. These findings are associated with an increased expression of Cx36 and Cx43, the two main connexins expressed in rat chromaffin cells. A stress-triggered upregulation of gap junctional coupling is similarly observed in mice. Indeed, Cx36 expression is enhanced in chromaffin cells of cold stressed mice, and as shown by in vivo experiments performed in anaesthetized mice, the uncoupling agent carbenoxolone more robustly reduces catecholamine secretion in stressed mice compared to controls. Collectively, these results demonstrate that in response to a physiological stress, gap junctionmediated intercellular communication between chromaffin cells positively contribute to the adrenal excitation-secretion coupling and ensuing catecholamine secretion. Strengthening the decisive role of connexin-dependent signaling in regulating stimulus-secretion coupling of the adrenal medullary tissue is our recent findings obtained in the pathological context of arterial hypertension, a pathology in which a sustained plasma catecholamine elevation can contribute to. Gap junction-mediated chromaffin cell communication was investigated in SHRs and compared to age-matched normotensive Wistar Kyoto (WKY) rats. In SHR acute adrenal slices, LY diffusion between chromaffin cells is significantly reduced, resulting in less that 20% of dye-coupled cells. This parallels a decrease in both Cx43 mRNA and protein, but not in Cx36. Although not yet completed by functional experiments (monitoring of junctional currents between chromaffin cell pairs), these results argue for a reduced gap junctional communication between chromaffin cells in chronically hypertensive rats. As such, depending on the physiological or pathological context, the adrenal medullary tissue dualistically and accurately adapts the competence of the gap junctional coupling between chromaffin cells. This reinforces gap junction signaling as a substantial component for neuroendocrine function in the adrenal medulla, as it may represent an additional lever regulating hormone release.

S60.2
PEPTIDERGIC CONTROL OF OXIDATIVE STRESS IN NEUROENDOCRINE CELLS

Youssef Anouar

Inserm, U982, Institute for Research and Innovation in Biomedicine, University of Rouen, Normandy University, Mont-Saint-Aignan, France

The mechanisms connecting tolerance to oxidative stress and energy supply to promote neuritogenesis and survival in neuroendocrine cells are largely unknown. We showed here that PACAP- and cAMP stimulate the expression of a novel antioxidant selenoprotein, named selenoprotein T (SelT), which exerts an essential role in tolerance against oxidative stress, cell survival and differentiation. By combining SelT promoter studies, RNA interference and ChIP analysis, we demonstrated that nuclear respiratory factor 1 (NRF-1), a key transcription factor regulating mitochondrial biogenesis, is crucial for SelT gene regulation by the cAMP/PKA pathway in PC12 cells. In addition, we showed that peroxisome proliferator-activated receptor γ coactivator-1α (PGC-1α), a potent NRF-1 coactivator involved in the transcriptional integration of mitochondriogenesis, is also required for cAMP/PKA-induced SelT gene transcription. Combined pharmacology, immunoblotting and knockdown experiments revealed that two critical kinases, LKB1 and AMP protein kinase (AMPK) operate downstream of PKA in order to promote PGC-1α expression in PC12 cells. Concurrently, using mitochondrial fluorescent tracking and determination of the ratio of mitochondrial to nuclear DNA, we found that PACAP and cAMP induced mitochondriogenesis in differentiating PC12 cells through a PKA/AMPK-dependent pathway. Taken together, these results show that a PKA/LKB1/AMPK/PGC-1/NRF-1 pathway couple tolerance to oxidative stress and mitochondrial biogenesis in response to peptidergic stimulation and cAMP elevation in neuroendocrine cells.

S60.3
FOOD INTAKE REGULATION IN RAT STRESS-INDUCED ANOREXIA

Fatiha Chigr

Biological Engineering Laboratory, Sultan Moulay Slimane University, Beni Mellal, Morocco; INRA Tours, François Rabelais University, Tours, France

Eating disorders are often attributed to deficits of metabolic regulation but little to brain deficits related to motivational problems. Thus, it was shown that stress can exacerbate motivational and eating disorders. However, the mechanisms of the pathophysiological changes in food intake (FI) regulation thresholds remain largely unknown, especially in the case of anorexia. The proposed investigations aim to analyze changes in brain levels of mRNA encoding neuropeptides: Proopiomelanocortin (POMC), cocaine amphetamine related transcript (CART), neuropeptide Y (NPY) and Agouti related peptide (AgRP) following the application of an acute stress (immobilization: IS) that causes anorectic behavior. Particular attention will be given to the hypothalamus and the dorsal vagal complex (DVC) the two main brain regions involved in FI. We showed, by using RT-PCR that in the hypothalamus, the mRNAs of POMC and CART were up-regulated at the end of IS and up to 24 h. This up regulation persists until 48–72 h after IS for CART only. In the DVC, their expressions peak significantly at 24 h post stress and decline afterwards; CART mRNA is down regulated after 48 h post stress. NPY and AgRP mRNAs show a gradual increase just after the end of IS. The up regulation is significant only at 24 h after stress for AgRP but remains significantly higher for NPY compared to controls. In DVC, the mRNAs of the two factors show generally a similar post stress pattern. A significant increase just after the end of IS of rats which persists up to 24 h after is firstly noticed. The levels tend then to reach the basal levels although, they were slightly but significantly higher up to 72 h after stress for mRNA NPY. The comparison between the expression profiles of anorexigenic and the two orexigenic peptides investigated shows the presence of a parallelism between that of POMC and AgRP and that of CART and NPY when each brain region (hypothalamus and DVC) is considered separately. It seems that any surge in the expression of each anorexigenic factor stimulates the expression of those of corresponding and appropriated orexigenic one. These last reactions from orexigenic peptides tend to attenuate the anorexigenic effects of CART and POMC and by consequent to abolish the anorexia state generated by stress. The changes in neuropeptides expression observed could reflect their involvement in adaptive behavior to stress.

S60.4
NEUROPEPTIDES IN STRESS SIGNALING FROM CELLS TO CIRCUITS IN THE CNS

Lee E. Eiden
Section on Molecular Neuroscience, NIMH-IRP, Bethesda, Maryland, USA

Neuropeptides involved in stress transduction, including pituitary adenylate cyclase-activating polypeptide, corticotropin-releasing hormone, ghrelin, and vasopressin, are synthesized as prohormones which are processed via proteolytic cleavage, C-terminal amidation, and post-translational modifications such as lipid acylation. Neuropeptides, in general, act post-synaptically via G-protein coupled receptors (GPCRs). There is variable evolutionary conservation of the primary sequences of neuropeptides; the processing 'punctuation' of their prohormones via basic amino acid flanking of the bioactive peptides; the amidating, acyl transferase, and other enzymes responsible for post-translational processing; and the cognate GPCRs through which they act. These evolutionary relationships, particularly the co-evolution of the peptides and their receptors, provide a point of persepctive, throughout insect, nematode, and vertebrate evolution, in which the dynamics of metazoan intercellular communication, during adaptation to the available ecological niches in which species have flourished, can be read. Indeed, mutational hot-spots identified by comparison of the genomes of h. sapiens and related genera include genes encoding neuropeptides, suggesting that variation in mammalian stress responding across species, and within a species, may have correspondences to differences, both subtle and marked, in the expression, distribution, and function of neuropeptides and their receptors. Neuropeptides are co-released with classical neurotransmitters at central and peripheral synapses mediating stress responses in vivo. From PACAP, a "master regulator" of the stress response centrally and peripherally, to vasopressin, a more specialized modulator of social behavior affected by stress, primordial neuropeptides have evolved from C. elegans to H. sapiens as integrators of environmental and interoceptive cues that control behavioral outputs. The prospects for exploiting neuropepeptidee receptor interactions for developing therapeuticcs for stress-related illnesses such as generalized anxiety disorder, major depressive disorder, and post-traumatic stress disorder, are considered from the standpoint of the anatomy of stress circuits and the neurochemistry of "stress synapses" controlling behavioral responses, including anhedonia, anxiety, and fear, in the hypothalamus, extended amygdala, and brain stem, using the PACAPergic system as a paradigmatic example. Development of specific pharmacological agents for both receptor-based and intracellular signaling-based attenuation of maladaptive responses to chronic and traumatic stress will be discussed.

S61 Room: Carlson Suite
ASTROCYTIC CONTRIBUTIONS TO SYNAPTIC FUNCTIONS

Chairs: Stéphane Oliet, FR and Dimtri Rusakov, UK

S61.1
RAPID ASTROCYTE MORPHOLOGY CHANGES SUPPORT EPILEPTIC ACTIVITY

Christian Henneberger
Institute of Cellular Neuroscience, University of Bonn Medical School Sigmund-Freud-Str. 25 53127 Bonn, Germany

Astrocytes closely contact neurons, which enables them to modulate and maintain neuronal function effectively by, for example, buffering potassium and glutamate clearance. A disruption of this spatial relationship could be of pathophysiological significance. Indeed, astrocyte dysfunction and long-term morphology changes have been implicated in numerous diseases including epilepsy. How rapid astrocyte morphology is altered by the onset of epileptiform activity and to what degree this contributes to aberrant network activity is largely unknown. Combining established protocols of hippocampal epileptogenesis, electrophysiology and two-photon excitation fluorescence microscopy allowed us to monitor astrocyte morphology changes during the induction of epileptiform activity in acute hippocampal slices. Analysis revealed that small and medium-sized astrocyte processes shrink acutely within minutes after epileptiform discharges appeared in the CA1 region. Importantly, similar astrocyte morphology changes were also detected 30 minutes after induction of status epilepticus in vivo by intracerebral kainate injection. In vitro, these astrocyte morphology changes outlasted the induction of epileptiform activity, persisted after pharmacological termination of epileptic activity by TTX and were sensitive to inhibition of Rho-associated protein kinase (ROCK, Y-27632). Interestingly, ROCK inhibition also reduced epileptiform activity, indicating that rapid astrocyte morphology changes support epileptic activity. A modification of glutamatergic or GABAergic synaptic transmission did not underlie the proconvulsive effect of astrocyte morphology changes. Instead, we observed that intracellular diffusion in astrocytes and diffusion between astrocytes via gap junctions were significantly decreased in parallel to morphology changes. The reduced astrocyte gap junction coupling is likely a consequence of reduced intracellular diffusion because no change of connexin 43 and 30 expression and phosphorylation was observed. Thus, astrocytes respond to epileptic activity with morphology changes on a time scale of minutes, which reduces intra- and intercellular diffusion in the astrocyte network and supports epileptic activity.

S61.2
ASTROCYTE SECRETION CAPACITY: FUSION PORE REGULATION

Robert Zorec
Laboratory of Neuroendocrinology-Molecular Cell Physiology, Institute of Pathophysiology, Faculty of Medicine, University of Ljubljana, Ljubljana, Slovenia; Celica BIOMEDICAL, Lab Cell Engineering, Ljubljana, Slovenia.

Astrocytes, a heterogeneous type of neuroglial cells, get excited when neurotransmitters bind to their membrane receptors by elevations in second messengers such as Ca^{2+} and cAMP. Activated astrocytes then signal their status to nighbouring cells, which involves vesicles. These store gliotransmitters or more generally gliosignaling molecules. In the former case chemical messengers get released from astrocytic sites proximal to the synapse, which defines communication to occur in the micro-space of contact between the synapse and the astrocyte. In contrast gliosignaling molecules may also be released into the extracellular space and get transported to locations far away from the active astrocyte. This mode of release resembles the endocrine system. Hence astrocytes are considered to be part of the gliocrine system in the brain, where the glymphatic system mediates the convection of released molecules. This complex system not only plays a role in cell-to-cell communication but also synchronizes the provision of energy for neural networks. Astrocytes contain glycogen, a form of energy store. Excitation of astrocytes by volume transmitters, such as noradrenaline (NA), released by locus coeruleus neurons, activates adrenergic receptors, excites Ca^{2+} and cAMP homeostasis and stimulatesaerobic glycolysis, providing lactate. This lecture will discuss how astrocytes operate to synchronize excitation and energy provision. Moreover, properties of Ca^{2+}-dependent fusion of a single vesicle with the plasma membrane in astrocytes will be presented, highlighting the role of SNARE proteins.

S61.3
ASTROCYTIC IP3 RECEPTORS AND THEIR ROLE IN SYNAPTIC PLASTICITY

Stephane Oliet
Neurocentre Magendie, Inserm 1215 and University of Bordeaux, Bordeaux, France

The role of astrocytic Ca^{2+} signaling in hippocampal long-term potentiation (LTP) is intensely debated. While Ca^{2+} release from astrocytic IP3 receptors (IP3R) have been suggested to regulate synaptic plasticity, genetic deletion of the putative astroctic IP3R, IP3R type 2 (IP3R2), does not impair synaptic plasticity. Here we report that IP3R2 is not the only functional IP3R subtype in astrocytes. Beneath the large IP3R2 mediated response, we revealed local monophasic Ca^{2+} events mediated by IP3R1 and IP3R3. On

testing if these newly discovered astrocytic Ca^{2+} channels regulate synaptic plasticity, we find that specific blockade of astrocytic IP3R1 impairs LTP. This demonstrates that the Ca^{2+} release channel IP3R1 regulates hippocampal LTP, resolving a long-standing debate, and highlighting the receptor specific role of local astrocytic Ca^{2+} dynamics in determining synaptic plasticity.

S61.4
DECIPHERING THE PRINCIPLES OF ASTROGLIAL SIGNALLING: EXPERIMENTS VERSUS THEORY

Dmitri Rusakov
UCL Institute of Neurology, University College London, UK.

Electrically non-excitable astroglia appear competent in propagating and integrating physiological messages using their internal communication medium of Ca^{2+} fluctuations. However, our understanding of the cellular machinery and dynamics of astrocyte Ca^{2+} signalling lags far behind the knowledge pertinent to signal integration in neurons. The advance of realistic biophysical models reproducing neuronal morphology and physiology has been crucial to our comprehension of nerve cells and their networks. There have hitherto been no systematic attempts to develop such models for astroglia, mainly because of the poor experimental accessibility of the three-dimensional, essentially nanoscopic organisation and activities of astroglia. Aiming at bridging this knowledge gap, we have adapted the NEURON simulation environment to construct a generic interactive model which can reproduce fine morphology, multi-faceted membrane properties and known signaling mechanisms of the typical protoplasmic astrocyte. The model enables distributed Ca^{2+} homeostasis mechanisms including Ca^{2+} diffusion and buffering, and the capability to recapitulate physiological observations in the three-dimensional nervous tissue. Exploring the case study of cortical astroglia, we have incorporated into the model a large set of morphological, electrophysiological and imaging data obtained in individual astrocytes *in vivo* and *in situ*. Our specific experimental observations have also enabled us to constrain the unknown model parameters thus revealing some basic features of astroglial membrane physiology and Ca^{2+} signalling, that have hitherto been inaccessible for direct probing. The present approach thus provides a novel and powerful tool to unravel astroglial physiology.

S62 Room: Ballroom
CAN ABSENCE SEIZURES BE PREVENTED?

Chair: Antoine Depaulis, FR

S62.1
BRAIN COMPUTER INTERFACES FOR ABSENCE SEIZURE INTERRUPTION AND PREVENTION

Annika Lüttjohann
Institute of Physiology I, Westfälische Wilhelms University Münster, Münster, Germany

An alternative treatment approach for epilepsy is deep brain stimulation, in which electrical pulses, aimed to modulate seizure activity, are delivered to a brain structure, either in a preprogrammed stimulation schedule (open loop stimulation) or triggered by the detection of a seizure in an on-line analysis of the EEG (closed loop stimulation). Rapid progress in the search for optimal stimulation parameters has been achieved in rat models of absence epilepsy. While open loop stimulation was rather ineffective in reducing the for absence epilepsy typical, bilaterally generalized spike and wave discharges (SWD), low intensity, closed loop, high frequency (130 Hz) stimulation was able to effectively disrupt ongoing SWD and shortened their duration. Closed loop stimulation induced also some neuroplastic changes considering that the reduction in SWD outlasted the stimulation period.

At the same time discovery of SWD precursor activity with the aid of advanced signal analytical techniques, which were applied to disentangle network mechanisms of SWD generation within the cortico-thalamic system, opened the way for the recent development of an online, SWD prediction algorithm and its implementation into a closed-loop, deep brain stimulation system aimed to predict and prevent the occurrence of SWD. The latter opens a new direction in the therapeutic strive for seizure freedom. It also demonstrates that, in contrast to long standing beliefs, SWD are not unpredictable in nature.

S62.2
MECHANISMS OF ANTIEPILEPTOGENESIS IN ABSENCE MODELS

Emilio Russo
Department of Science of Health, School of Medicine and Surgery, University of Catanzaro, Catanzaro, Italy

The lack of antiepileptogenic therapies is one of the major unmet needs on epilepsy research, despite the availability of many animal models. To this aim, animal models have been the major source of our current understanding and have led to the identification of potential targets for epilepsy development prevention and/or progression; however, none of the currently available models

has been clinically validated. New insights are now coming from so-called genetic animal models which are being reconsidered as potential models to study the pathophysiology of the epileptogenic process (differently from acquired/post-insult models) and the potential antiepileptogenic efficacy of drug treatments. Our current knowledge suggests that some processes (e.g. neuroinflammation) might be in common in the epileptogenesis that occurs in post-insult models and genetic models; however, some important differences also exist (e.g. no clear neurodegeneration, neurogenesis) and also differences might well be observed between different genetic models.

In WAG/Rij rats, the first seizure appears about the age of 2 months; therefore, we hypothesize that the period of life before seizure appearance in this strain, as well as other genetic models, might be considered the corresponding latent phase (period) studied in post-insult models. Brain insults such as brain injury or stroke are clearly identifiable events, while in WAG/Rij rats, altered genes might be considered as the initial 'insult' leading to the development of spontaneous seizures. Indeed, this theory needs to be validated and further studies are warranted to better understand and identify differences between epileptogenic processes in genetic and post-insult models.

Comparing WAG/Rij rats with what is known for post-insult models, we found that: 1) there is not yet a clear demonstration of an involvement of neuroinflammation in epileptogenesis; it cannot however, be excluded that local inflammatory processes are involved, as observed for GAERS. The currently published articles confirm that no major inflammatory processes are undergoing before and after seizure appearance; 2) no neurodegeneration is observed in any brain area of these rats before seizure onset as well as any other major brain abnormalities. Both these points clearly differentiate WAG/Rij rats from other post-insult models, while mTOR hyperactivation seems to be a common link with the latter. Furthermore, several alterations have been identified in the glutamatergic systems, which are, the most studied in this strain, while considering pre-seizure age. Finally, some relevant alterations are observed in the function of several ion channels, leading to hyperexcitability, which, together with possible changes in endocannabinoids, deserve further confirmatory and explanatory studies. A variety of drug treatments have already been tested in this strain and while antiseizure efficacy was demonstrated for some of them, we critically observed that in some studies, drug effects were not persistent and seizures tended to reappear sometimes after drug withdrawal. Drug testing protocols for this strain and possibly other genetic models should therefore be structured to consider longer-lasting follow-up seizure detection after drug treatment suspension. In conclusion, we propose that WAG/Rij rats and other genetic epilepsy models represent a unique opportunity to increase our knowledge on epileptogenesis; future studies should consider earlier life periods and should be aimed at the identification of early processes that could be targeted for future drug therapies.

S62.3
EXPLORING THE INITIATION SITE OF EXPERIMENTAL ABSENCE SEIZURES: NOVEL PATHWAYS FOR PATIENT-TAILORED TREATMENT

Mojam Carcak

Neuroscience Division, School of Biosciences, Cardiff University, Cardiff, UK; Department of Pharmacology, Faculty of Pharmacy, Istanbul University, Istanbul, Turkey; Department of Pharmacology and Clinical Pharmacology, Marmara University School of Medicine, Istanbul, Turkey

Absence seizures are accompanied by spike-and-wave discharges (SWDs) in cortical electroencephalogram. SWDs consist of bilateral 3–4 Hz discharges driven by synchronized oscillatory activity in reciprocally connected cortical and thalamic networks. Investigations in both human patients and genetic rat models of absence epilepsy has shown that SWDs have a cortical focal onset. Experimental work has indicated that SWDs originate from a population of cortical neurons located in layers 5 and 6 of the peri-oral somatosensory cortex before they spread to other cortical areas and to thalamic territories. A similar observation has been made in children with absence epilepsy, where the existence of a putative cortical initiation site was found mostly in frontoparietal areas. Interestingly, different children show different initiation sites within this cortical area, but in the same child the position of the initiation site remains unaltered across seizures. In line with these results, in experimental animals, the cortical initiation site can vary across species and models of absence epilepsy. In Stargazer mice, the initiation site has been found to be in the motor cortex and not in the somatosensory cortex as in the other mice models (GAT-1 knock-out mice and pharmacological γ-hydroxybutyrate and pentylentetrazole mice models) and rat genetic models of absence epilepsy (GAERS and WAG/Rij). Moreover, using non-linear dynamic analysis in these different models we found that the initiation site and the area where each individual spike and wave cycle initiates could differ. Thus, for example in Stargazer mice the motor cortex is the initiation site of the ictal discharge but the parietal cortex is the region where each spike and wave cycle starts, suggesting a diversity of mechanisms which have often been considered similar across absence epilepsy models. These findings highlight the possibility

that different underlying abnormalities may lead to different sites of initiation and suggest that accurate analysis of SWD initiation in patients needs to be carried out before any external intervention for the prevention and/or treatment of absence seizures.

S62.4
ON THE FEASIBILITY OF SEIZURE CONTROL AND PREVENTION IN ABSENCE PATIENTS

Cian McCafferty
Departments of Neurology, Neuroscience and Neurosurgery Yale University School of Medicine, New Haven, CT 06520-8018 USA

Absence seizures are a common and disabling seizure type associated with impaired consciousness during seizures and chronic comorbidities in the interictal period. Recent work from experimental animal models suggests that early intervention with effective medical treatment can have disease-modifying effects and improves long-term outcome. We sought to determine whether similar early intervention may prevent absence epileptogenesis in human patients. Absence epilepsy prognosis was investigated through two different approaches. One involved a longitudinal study of outcome in children living in Connecticut diagnosed with absence epilepsy. Medication treatment and long term outcome was analyzed in this group. The second approach was a retrospective meta-analysis of published studies that included data on medication treatment and long-term seizure freedom off medications. We found in both the longitudinal study of children in Connecticut and in the retrospective meta-analysis of published data that treatment with ethosuximide was associated with improved long-term seizure-free prognosis off medication.

S62.5
BUILDING UP ABSENCE SEIZURES: NETWORK AND CELLULAR PROCESSES OF ABSENCE EPILEPTOGENESIS

Guillaume Jarre
Univ. Grenoble Alpes, Grenoble Institut des Neurosciences, GIN, Inserm, U1216, F-38000 Grenoble, France

Absence Epilepsy (AE) is most often diagnosed during childhood and this early onset suggests that epileptogenesis process takes place during brain development and maturation. Nevertheless, little is known about the cellular and network dysfunctions leading to the emergence of recurrent seizures. As one major challenge in epilepsy research is to find and develop strategies to prevent epilepsy, understanding of the epileptogenesis process is needed to find an appropriate time windows for anti-epileptogenic treatment.

We investigated *in vivo* the network and single neuron mechanisms responsible for the emergence of epileptic activity in GAERS using local field potential (LFP) follow-up and intracellular recordings of cortical somatosensory neurons. We then tested the effect of a chronic treatment with anti-absence drugs before the emergence of spike-and-wave discharges (SWD). LFP recordings showed that immature cortical discharges progressively evolved into typical SWDs following a three-step maturation process. Intracellular recordings revealed that this maturation was accompanied with an evolution of neuronal intrinsic properties, associated with a growing propensity of neurons to generate synchronized oscillations. Finally, daily administration of ethosuximide or valproate before SWDs occurrence did not alter epileptogenesis or the drugs efficiencies at adulthood.

Our data suggest that oscillatory cortical discharges are the first clinical sign of absence epileptogenesis and that the maturation process of SWD results from a progressive propensity of the neuronal network to generate synchronized oscillations. We found that chronic treatments with anti-absence drugs before SWD onset did not alter epileptogenesis. This suggests that current treatments are not appropriate to alter or reverse epileptogenesis when they are administrated before seizures onset.

S63 Room: Clermont Suite
FRAGILITY OF DECLARATIVE MEMORY: INSIGHT FROM DIFFERENT ANIMAL MODELS

Chair: Marighetto Aline, FR

S63.1
JUVENILE STRESS AND HIPPOCAMPAL FUNCTION

Gal Richter-Levin
Sagol Department of Neurobiology, University of Haifa, Haifa, Israel

Exposure to a brief but significant stress in childhood is a well-known risk factor for developing stress-related psychopathologies later in life. Stress-related psychopathologies, such as post-traumatic stress disorder (PTSD) are considered to be associated with alterations in hippocampus volume and functions, but the exact neural mechanisms behind that are yet to be studied. We have developed an animal model of childhood stress – the post-weaning, pre-pubertal (Juvenile) stress model, which now enables us to address those questions. In a series of experiments we have first demonstrated that indeed, exposure to juvenile stress results in impaired ability to learn under stress and increased vul-

nerability to stress in adulthood. Most studies looking at the effects of stress on the hippocampus focus on the Cornu Ammonis areas, mainly CA1 and CA3. We found evidence for a unique role of the dentate gyrus (DG) under emotional and stressful conditions. Examining the impact of juvenile stress exposure on DG activity we have identified selective alterations of GABAergic interneurons-granule cells interactions, suggesting that GABA-associated proteins are involved. However, not all individuals respond to stress in a similar way. We have developed a 'Behavioral Profiling' approach, which enables us to differentiate between exposed-affected and exposed-nonaffected individuals. Employing this novel approach we found that some of the GABA-related alterations within the DG are associated with stress vulnerability but others are associated with developing stress resilience.

S63.2
CELLULAR BASES OF TEMPORAL BINDING ASSESSED IN TRACE FEAR CONDITIONING IN MICE

Azza Sellami
Faculté des Sciences de Tunis, Neurophysiologie fonctionnelle et pathologies, Tunis 2092, Tunisia; Inserm u862, Neurocentre Magendie, Pathophysiology of Declarative Memory Group, Bordeaux-F33077, France

Cognitive aging involves the prominent deterioration of declarative memory. Development of therapeutic approaches to cognitive aging may improve identifying processing mechanisms underlying this memory and their neurobiological bases. Previous work identified temporal binding, the capability to associate temporally distant stimuli, as a critical process for remembering complex associations among events, i.e. declarative memory. The CA1 subfield of the hippocampus is involved in memory of temporal associations, but its function remains to be specified. Based on the discovery of time cells, which fire at successive moments in temporally structured experiences (McDonald et al., 2011; Eichenbaum H., 2013), an hypothesis was recently proposed: activity of CA1 cells would bridge the gap in memory for discontiguous events. To test this hypothesis, first, we confirmed that CA1 activity is related to successful temporal binding in memory by combining trace fear conditioning procedure at different levels of temporal binding demand (tone-shock interval length) to Fos neuroimaging. We found that successful tone-shock binding in memory only occurs for a tone-shock interval of 20 seconds and that CA1 is the sole area in which activity is increased by successful temporal binding compared to the other learning conditions under which no temporal binding occurs. Then we demonstrated that CA1 activity is necessary during temporal gaps between the (to be-associated) stimuli for successful temporal binding by combining trace fear conditioning to an optogenetic approach. We inhibited CA1 activity specifically in or out of the trace interval during acquisition of 20 seconds trace conditioning. We found that successful tone-shock binding in memory requires CA1 activity during temporal gap between the tone and the shock. In conclusion, our findings validate the "time cells" hypothesis that CA1 activity is critically needed during learning to bridge temporal gaps between discontiguous events in memory.

S63.3
A DEFICIT IN DECLARATIVE/CONTEXTUAL MEMORY: BASIS OF TRAUMATIC MEMORY

Aline Desmedt
Neurocentre Magendie, INSERM U1215, Université de Bordeaux, France

For over a century, clinicians have described a *qualitative alteration* of traumatic memory, which corresponds to a paradoxical co-existence of *hypermnesia* and contextual *amnesia* for the same event. However, in basic neuroscience, most current animal models have exclusively focused on the hypermnesia: the persistence of a strong fear memory, which, yet, can be *adaptive per se*. Because they do not distinguish between normal and abnormal stress-induced biobehavioural changes, the stress-related changes observed can be either aspects of the PTSD phenotype or normal/adaptive responses to stress. It is, thus, necessary to define the boundary conditions in which a stress condition leads to PTSD or not. An appropriate experimental design should allow the systematic comparison between *adaptive* and *maladaptive* fear memory, in order to address the following key issue: how to switch from *normal to pathological* fear memory, with the perspective to restore a normal fear memory once a PTSD-like fear memory would have been established. Recently, we developed a model for PTSD-related memory in mice that precisely allows the direct comparison between "normal" vs. traumatic (i.e. "PTSD-like") fear memory. On this basis, I will present recent findings identifying specific cellular and molecular alterations within the hippocampal-amygdalar network underlying the development of PTSD-like memory. These specific neurobiological alterations may constitute new therapeutic targets in the treatment of this stress-related disorder.

S63.4
DEFECT IN TEMPORAL BINDING IS THE SOURCE OF AGING-RELATED DECLINE IN DECLARATIVE MEMORY

Nicole Etchamendy
Neurocentre Magendie Inserm U1215, Université de Bordeaux, 146 rue Léo Saignat 33077 - Bordeaux – France

One major component of cognitive aging is the decline of declarative memory. Here, we study temporal binding, the process by which discrete stimuli can be associated in memory despite their temporal separation, and its relationships with another fundamental function of the hippocampus: relational organization. Relational organization links independently acquired memories via common elements and consequently supports cardinal flexibility of declarative memory. Both these processes are known to deteriorate in aging, but potential links between them remain hypothetical. First, using mice, we demonstrate that temporal binding relies on CA1 activity. Second, we identify a causal link between CA1-dependent temporal binding and relational organization sustaining characteristic flexibility of declarative memory. Namely, temporal binding is a necessary condition for building a relational representation that allows flexible expression of memories. In aging, we discovered that a reduction of temporal binding capacity is the primary cause of the relational/declarative memory impairment. Indeed, in both mice and humans, relational/declarative memory per se is not impaired during aging, since this memory remains intact in aged individuals as long as the temporal proximity between events minimizes the temporal binding demand. In conclusion, our findings identify the bridging of temporal intervals sustained by hippocampal CA1, as a critical determinant of declarative memory formation, and demonstrate that the age-related decline in this hippocampal function is the primary source of the memory loss.

S64 Room: Clermont Suite MONITORING BRAIN INFLAMMATION

Chairs: Galila Agam, IL and Sara Eyal, IL

S64.1 TRACKING INFLAMMATION IN THE EPILEPTIC RAT BRAIN BY BIFUNCTIONAL FLUORESCENT AND MAGNETIC NANOPARTICLES

Sara Eyal
Institute for Drug Research, School of Pharmacy, The Hebrew University of Jerusalem, Jerusalem, Israel

Correct localization of epileptic foci can improve surgical outcome in patients with drug-resistant seizures. Using the rat lithium-pilocarpine model of temporal lobe epilepsy, we have recently shown that systemically injected nanoparticles identify activated immune cells, which have been reported to accumulate in epileptogenic brain tissue. The nanoparticles were detected by both confocal microscopy and by MRI. However, it was not clarified whether the nanoparticles were delivered to epileptogenic brain tissue by systemic macrophages or locally uptaken, by resident immune cells. This issue is relevant not only for potential localization of pathological brain tissue to be resected and for drug delivery, but also for understanding the basic mechanisms underlying activation of the innate immune system in the epileptic brain. In this talk we will discuss our recent work aimed to address these issues.

S64.2 PSYCHOTROPIC DRUGS ATTENUATE LIPOPOLYSACCHARIDE-INDUCED HYPOTHERMIA BY ALTERING HYPOTHALAMIC LEVELS OF INFLAMMATORY MEDIATORS

Abed Azab
Department of Nursing and Department of Biochemistry and Pharmacology, Faculty of Health Sciences, Ben-Gurion University of the Negev; Beer-Sheva, 84105, Israel

A large body of data suggests that inflammation plays a role in the pathophysiology and treatment of mood disorders. Consistently, psychotropic drugs have been shown to have potent anti-inflammatory effects, and, classic anti-inflammatory drugs were found to exert therapeutic benefits in randomized clinical trials in patients with mood disorders. 1) We investigated the effects of several mechanistically different psychotropic drugs on lipopolysaccharide (LPS)-induced inflammation in rat primary glia cells in-vitro and in rat brain *in vivo*. 2) We examined the efficacy of typical anti-inflammatory drugs in behavioral models in rats. Protocol 1: Rat primary glia cells were treated with different psychotropic drugs and then with LPS, and culture medium was collected for determination of inflammatory mediators' levels. Protocol 2: Rats were treated with psychotropic drugs for 28 days through a single daily intraperitoneal (ip) injection. On day 29, rats were injected (ip) with saline or LPS. At $\sim$ 2 h post LPS injection rats were sacrificed, blood was collected and different brain regions were excised. Levels of inflammatory mediators in glia culture medium, plasma and brain samples were determined by specific ELISA kits. Protocol 3: Rats were treated (ip) with four mechanistically different anti-inflammatory drugs for 2 weeks. At the end of drug treatment animals were subjected to behavioral tests relevant to the study of mood disorders. We found that psychotropic drugs possess both anti- and pro-inflammatory effects. Some of the drugs prominently affected the levels of particular inflammatory mediators in the plasma and specific brain regions, raising

the possibility for the development of mechanistic- and region-directed therapeutic interventions. Moreover, we found that anti-inflammatory drugs affected the behavioral phenotype of rats subjected to various behavioral models. These data add to the existing evidence suggesting that inflammation plays a role in the pathophysiology and treatment of mood disorders. Examining the therapeutic potential of mediator-specific anti-inflammatory compounds will be of high scientific value in order to establish anti-inflammation as a treatment strategy for mood disorders.

S64.3
LITHIUM AS AN ANTI-NEUROINFLAMMATORY AGENT

Galila Agam

Department of Clinical Biochemistry and Pharmacology; Psychiatry Research Unit; Faculty of Health Sciences; School for Community Health Professions; Ben-Gurion University of the Negev and Mental Health Center, Beer-Sheva, Israel

Neither the etiology/physiopathology of bipolar disorder (BD, manic depressive illness) nor the mechanism of mood stabilization have been unraveled to date but recent studies have suggested that they might be related to immune system alterations and inflammation. Indeed, BD is closely associated with medical comorbidities, such as cardiovascular disease, diabetes mellitus, obesity and thyroid dysfunction and the inflammatory disturbances frequently observed in BD could explain, at least in part, these comorbidities.

Cytokines play an important role in inflammatory signaling and regulate both the innate and adaptive immune responses. In addition, cytokines also play roles in neurotransmitter metabolism, neurogenesis and the neuroendocrine system. Studies have revealed alterations in peripheral markers of inflammation, such as the cytokines interleukin (IL)-1β, IL-2, IL-4, IL-6, IL-10, and tumor necrosis factor-alpha (TNF-α), in patients with BD.

Lithium (Li) is considered the first-line agent for both acute mania and maintenance treatment of this mood disorder. Li has been implicated to exhibit anti-inflammatory effects that may contribute to its therapeutic efficacy but the mechanism mediating the anti-inflammatory effects remain unidentified. We have previously shown that homozygote knockout (KO) of inositol-monophosphatase1 (IMPA1) results in lithium (Li)-like behavior. We now hypothesized that Li-treated mice and IMPA1 KO mice exhibit a similar brain cytokines profile. Hippocampal, frontal cortex and hypothalamic cytokine levels were measured in three groups of mice: wildtype (WT) on regular-food, WT on Li-supplemented food and IMPA1-KOs. The effect of Li and of IMPA1 KO on cytokine levels differed among the three brain areas studied. Only in the hippocampus both interventions exerted similar effects. Frontal cortex cytokine levels were unaffected neither by Li nor by IMPA1 KO. This suggests that the mechanism mediating Li's effect on the inflammatory system differs among brain regions. Only in the hippocampus the results favor the involvement of the phosphatidylinositol (PI) cycle.

S64.4
NEW MODALITY OF GSK-3 INHIBITION HOLDS PROMISE IN TREATING NEURODEGENERATIVE DISORDERS

Hagit Eldar-Finkelman

Department of Human Molecular genetics and Biochemistry, Sackler School of Medicine, Tel Aviv University, Israel

Glycogen synthase kinase-3 (GSK-3) has emerged as an important drug discovery target in treating Alzheimer's disease (AD) and related neurodegenerative disorders. Mechanisms linking GSK-3 with AD include phosphorylation of tau protein, activation pro-inflammatory factors; accelerated processing of amyloid precursor protein (APP); and inhibition of cellular clearance pathways including autophagy and lysosomal function. Inhibition of GSK-3 is thus a promising therapy for treating these disorders. Most GSK-3 inhibitors have not reached the clinic, however, in large part due to limited specificity and resulting toxic effects.

We postulated that 'correct' inhibition of GSK-3 is a key for a successful treatment. Our laboratory is focused on developing compounds that specifically bind to the unique substrate binding site of GSK-3, this to insure specificity. Recently we identified a novel mechanism of inhibition that converts substrate into a very potent and highly selective inhibitors. Treatment with our inhibitor L807mts enhanced the clearance of beta amyloid loads, reduced inflammation, enhanced autophagic flux, and improved cognitive and social skills in the 5XFAD AD mouse model. L803mts also shows reasonable pharmacological and safety properties when tested in rodents.

Based on the properties of L807mts we developed additional inhibitors termed L808mts and L809mts that are very potent GSK-3 inhibitors with $IC_{50} < 1\,\mu M$. *In vivo* studies confirmed their biological properties, and, in addition treatment with L809mts improved anxiety and mood behavior in a mouse model. We suggest that this unique modality of GSK-3 inhibition brings new opportunities in development of potent and highly selective GSK-3 inhibitors for future therapy.

S65 Room: Marie Louise 1
FOOD INTAKE AND ENERGY METABOLISM CONTROL: PHYSIOLOGICAL AND PHYSIOPATHOLOGICAL ASPECTS

Chairs: Nicolas Chartrel, FR and Mohamed Najimi, MA

S65.1
26RFA: A NEUROPEPTIDE INVOLVED IN THE HYPOTHALAMIC REGULATION OF FOOD INTAKE AND GLUCOSE HOMEOSTASIS

Nicolas Chartrel
INSERM U982, Laboratory of Neuronal and Neuroendocrine Differentiation and Communication, Institute for Research and Innovation in Biomedecine (IRIB), Mont-Saint-Aignan, France; Normandy University, Caen, France; University of Rouen, Rouen, France

26RFa, a hypothalamic neuropeptide discovered by our team, was identified as the endogenous ligand of an orphan human receptor, GPR103. 26RFa strongly stimulates food intake. 26RFa is up-regulated in obese animal models and its orexigenic activity is accentuated in rodent fed a high fat diet, suggesting that this neuropeptide might play a role in the development and maintenance of the obese status. Obesity and type II diabetes arefrequently associated. Their significant and synchronous progression for 30 years so that the pandemic "diabesity" currently observed worldwide, is a real public health issue.

Recent studies revealed that neuropeptides known to play a crucial role in the hypothalamic control of feeding behavior are also expressed in pancreatic islets, suggesting that hypothalamic neuropeptides could provide the link between energy and glucose homeostasis, and constitute potential therapeutic targets for the treatment of obesity associated with type II diabetes. Indeed, our human studies showed a moderate positive correlation between plasma 26RFa levels and plasma insulin in diabetic patients. Plasma 26RFa concentration also increases in response to an oral glucose tolerance test. In addition, we found that 26RFa and its receptor GPR103 are present in human pancreatic β cells as well as in the gut. In this context, we investigated whether 26RFa may be involved in the regulation of glucose homeostasis in mice. We found that 26RFa attenuates the hyperglycemia induced by a glucose load, potentiates insulin sensitivity and increases plasma insulin concentrations. Consistent with these data, 26RFa stimulates insulin production by MIN6 insulinoma cells. Finally, we show, using *in vivo* and *in vitro* approaches, that a glucose load induces a massive secretion of 26RFa by the small intestine indicating that 26RFa acts as an incretin to regulate glucose homeostasis. Recent studies investigating a dysfunction of the 26RFa/GPR103 system in diabetes will also be presented.

S65.2
NUTRITIONAL LIPIDS AND BRAIN INFLAMMATION: A NEW THERAPEUTIC PATHWAY FOR THE TREATMENT OF OBESITY?

Carole Rovere-Jovene
Université Côte d'Azur, INSERM, CNRS, IPMC, France

Sickness behavior defines the endocrine, autonomic, behavioral and metabolic responses associated with infection. While inflammatory responses were suggested to be instrumental in the loss of appetite and body weight, the molecular underpinning remains unknown. In this study, we show that systemic or central lipopolysaccharide (LPS) injection results in specific hypothalamic changes characterized by a precocious increase in the chemokine ligand 2 (CCL2) followed by an increase in pro-inflammatory cytokines and a decrease in the orexigenic neuropeptide melanin-concentrating hormone (MCH). We therefore hypothesized that CCL2 could be the central relay for the loss in body weight induced by the inflammatory signal LPS. We find that central delivery of CCL2 promotes neuroinflammation and the decrease of MCH and body weight. MCH neurons express CCL2 receptor and respond to CCL2 by decreasing both electrical activity and MCH release. Pharmacological or genetic inhibition of CCL2 signaling opposes the response to LPS at both molecular and physiologic levels. We conclude that CCL2 signaling onto MCH neurons represents a core mechanism that relays peripheral inflammation to sickness behavior.

S65.3
ANOREXIA-INDUCED STRESS INVOLVED IN NEUROCHEMICAL AND MORPHOLOGICAL CHANGES

Mohamed Najimi
Biological Engineering Laboratory, Sultan Moulay Slimane University, Beni Mellal, Morocco and INRA, F. Rabelais University, Tours, France

It is now well established that the production of new neurons occurs in several regions in adult mammalian brain and is not restricted to the embryonic and early postnatal periods. This neurogenenic activity has been demonstrated to integrate functional neuronal circuits as in the hippocampus, the most characterize brain area. Other structures like olfactory bulb-subventricular zone: OB-SVZ complex, and recently the hypothalamus and dorsal vagal complex: DVC in the brainstem have been demonstrated to display neurogenesis in adult brain

too. Interestingly, all these structures are involved indirectly or directly in food intake: FI regulation and decision making to eat. Furthermore, neurogenesis as FI are very sensitive to stressful events. Indeed, different stress paradigms have been reported to affect negatively neurogenesis in hippocampus and lead to the setting of anorexia in rodents. To what extent does stress affect neurogenesis in the other neurogenic structures in relation to FI regulation, this is the goal of the present work. Using an homotypic and predictable immobilization stress (1 h immobilization daily during 3 weeks) in adult male rats, we found that neurogenesis is reduced by 30% in DVC taken at whole with differential in its components. Area postrema is more affected that nucleus tractus solitaire and dorsal motor nucleus of vagus. An equivalent reduction rate in neurogenesis is also observed in hippocampus. In OB-SVZ, an opposite pattern was shown, as a high increase in neurogenic activity was evidenced in stressed rats. Behavioral tests have showed that stressed rats display decrease in body weight gain and FI (anorexia-like state) which could correlated to the decrease in neurogenesis in DVC, one key region involved in FI regulation. The anxious developed by stressed rats could also be correlated with reduced hippocampal neurogenesis. The increase of OB-SVZ neurogenesis could be explained as a survival strategy of stressed rats to counteract anorexia state developed by enhancing capacity of smelling food. Indeed, using behavioral odor tests, we showed that the capacity of stressed rats to smell food was highly enhanced compared to control and arguing indirectly that the newborn euros could effectively be functional.

S65.4
IS THE PLASTICITY OF BRAIN FEEDING CIRCUITS INVOLVED IN SATIETY?

Alexandre Benani
Centre des Sciences du Goût et de l'Alimentation, CNRS, Dijon, France

Changes in synaptic connection within neuronal circuits controlling appetite and metabolism may occur in the adult brain. Yet, little is known about this property and its exact role in the maintenance of energy homeostasis, except that state-dependent synaptic plasticity is coordinated by hormonal signals across multiple neuronal cell types dispersed in the hypothalamus. For instance, synaptic plasticity in AgRP and POMC neurons of the melanocortin system has been evidenced in response to short-term overfeeding and 24-hr fasting. Both are extreme metabolic conditions associated to large hormonal changes. Thus, we still do not know whether such plasticity is recapitulated in response to moderate hormonal fluctuation just as it happens at the meal scale. In recent works, we used electron microscopy, confocal imaging and electrophysiology to deconstruct neuro-glial interaction in anorectic POMC neurons from Tomato-tagged mice following a meal. We found significant synaptic and neuro-glial changes on POMC neurons according to the nature of ingested food. Our results indicate that neuro-glial plasticity of the melanocortin system is highly reactive to changes in the metabolic state.

S66 Room: Marie Louise 2
UNDERSTANDING AND PROTECTING PROGRESSIVE HEARING LOSS

Chair: Isabel Varela Nieto, ES

S66.1
TREATMENT OF PROGRESSIVE HEARING LOSS WITH HEARING PROSTHESIS

Rami Saba
MED-EL, Innsbruck, Austria

Despite hearing loss being marginalized in confrontation with other sensory deficits such as blindness due to its milder disabling conditions, its different forms and levels of severity have been successfully targeted with biomedical engineering approaches and several solutions are currently clinically available for patients suffering from social and economic exclusion due to such disability.

Everyone is familiar with the fact that mild forms of deafness are sufficiently treated with properly fitted hearing aids. However, there are nowadays treatment solutions also for sever to profound levels of deafness (hearing threshold of 60 dB and above) unimaginable before. Nevertheless, such treatment requires a surgical approach in which a hearing prosthesis, cochlear implant (CI) is implanted into the inner ear of the patient. In the specific case of progressive hearing loss, this typically implies implanting a long electrode array which extends along the whole length of the cochlea of the inner ear. Such an electrode array is able to deliver an electrical signal to stimulate different regions of the auditory nerve corresponding to different frequency bands, creating the perception of sound even for the profoundly deaf.

The need for the "full cochlear coverage" with a long electrode derives from the fact that even if the patient at the moment of implantation has significant acoustic hearing (typically at low frequencies) and electrical stimulation would be sufficient only at relatively shallow cochlear locations (tuned to higher frequencies), such residual hearing can be lost with time. Patients implanted with shorter (and less traumatic electrodes) would be in the future deprived of low frequency sound in their elec-

trical hearing, essential not only for speech comprehension, but also for spatial orientation based on acoustic cues.

The fundamental step in treatment of severe to profound forms of deafness is therefore the pre-implantation diagnosis of hearing loss stability for each CI candidate. In the case that the hearing is stable enough and the patient has significant (low frequency) residual hearing, a cochlear implant with a shorter electrode is selected. It will provide electrical hearing at higher frequencies, while minimal trauma associated with its implantation, will guarantee long-lasting acoustic low frequency hearing. If necessary, the acoustic hearing can be amplified in parallel by a hearing aid. This hearing solution is called Electric-acoustic stimulation (EAS). In the case that the hearing is not stable and degrades over time, the long electrode needs to be used to electrically stimulate the whole frequency region essential for sound perception (0.2–10.0 kHz), which would be the range of a standard cochlear implant.

The stability of residual hearing is traditionally measured acoustically by an audiologist. This approach however is time consuming, requires several measuring sessions, which are often difficult to attend by patients and therefore, not very satisfactory. New, more (time) effective, diagnostic alternatives are therefore needed. One promising approach is the use of genetic markers of progressive hearing loss. In fact it has been established that mutations in some specific genes are highly associated with progressive hearing loss. For example, TMPRSS3 and TMC1 genes mutations are now know to be associated with progressive hearing loss and require the long electrode array implantation. Also mutations in the seed region of MicroRNA-96 and in the ATP-gated P2X2 receptor are responsible for progressive hearing loss. On the other hand, CDH23, GJb2, Gjb6, LOXHD1 and mitochondrial genes mutations are known to lead to stable deafness, for which EAS approach is more suitable. Further developments in the interface between genetics and hearing sciences are expected to further improve the benefits of hearing implants to the patients.

S66.2
SYNTHETIC OXYSTEROLS ARE NOVEL HEARING PROTECTIVE DRUGS

Philippe De Medina
AFFICHEM, Toulouse, France

Sensorineural hearing loss (SNHL) is a major pathology of the inner ear that affects nearly 600 million people worldwide. Age-related sensorineural hearing loss is the second most common disability of the elderly affecting about half of the population over 75 years old. SNHL remains nowadays without satisfactory solutions underlying the strong expectation toward innovative therapeutics. Spiral ganglion neurons (SGN) are major players in hearing conveying electrical signal from cochlear sensory cells (hair cells) to the central auditory pathway. Since SGN degeneration has been extensively described as a cause of hearing loss, neuroactive compounds are potential therapeutic drugs for SNHL. We have discovered a new family of neuroactive cholesterol derivates called Dendrogenins. These latter display potent activities both *in vitro* and in animal models of hearing loss. Dendrogenins are promising drug candidates for the prevention and treatment of hearing loss opening new potential opportunities for patients suffering from this pathology.

S66.3
AGE-RELATED ALTERATIONS IN THE CENTRAL AUDITORY PATHWAY

Jiri Popelar
Institute of Experimental Medicine, Czech Academy of Sciences, Czech Republic

Age-related impairment, or presbycusis, occurs in everyone, but to a different extent. Presbycusis is a multifactorial process, with impairment induced by noise exposure, ototoxic drugs, inflammation, and genetic factors. Pathological processes such as apoptosis and necrosis, damage the hair cells of the inner ear as well as the neurons of the spiral ganglion. However, significant alterations are also present in the central auditory system, including the auditory cortex. In experiments with rats we found that the number of neurons in individual nuclei of the central auditory system does not decrease significantly with aging, however significant losses occur in the number of special types of neurons, such as neurons containing non-phosphorylated neurofilaments (SMI-32), GAD-immunoreactive inhibitory neurons, parvalbumin, calbindin and calretinin immunoreactive neurons, and related proteins. The results of electrophysiological and behavioral experiments in animals demonstrate an age-related decline in the processing of temporal parameters of acoustical signals. In humans, the most known are age-related increases in the hearing thresholds, that first affect high-frequency hearing, and spread with aging, also to low frequencies. However, presbycusis is also characterized in humans by a deterioration of speech understanding, especially in loud backgrounds, and a deterioration of space hearing. Magnetic resonance imaging offers new insights into the changes of the human central auditory system with aging. MR spectroscopy shows an age-related decline in some metabolites in the auditory cortex, such as n-acetyl-aspartate or glutamate. MR morphometry demonstrates age-related decreases in the width of the auditory cortex (that are in principle not influenced by

the level of the peripheral hearing loss), and preserved differences between the parameters of the left and right auditory cortices. The results of functional MRI inform us about the larger activation of the right non-dominant auditory cortex in the processing of acoustical signals in the elderly. Changes in the central auditory system with the aging process must be taken into account when considering successful rehabilitation and repair of hearing in the elderly.

S66.4
GENETIC REDUCTION OF INSULIN-LIKE GROWTH FACTOR LEVELS EXACERBATES OTIC DAMAGE VIA ACTIVATION OF JNK SIGNALING AND UPREGULATION OF PRO-INFLAMMATORY CYTOKINE PRODUCTION

Isabel Varela-Nieto
Instituto de Investigaciones Biomédicas "Alberto Sols" (CSIC-UAM), Madrid, Spain

The onset and progression of Age-Related Hearing Loss (ARHL) depends on genetic and environmental factors not entirely well-characterized. The homozygous deficiency in insulin-like growth factor 1 (IGF1) causes poor growth rates, mental retardation and syndromic HL. Accordingly, low levels of IGF-1 have been associated with HL in related human genetic syndromes. Furthermore, circulating IGF-1 levels decrease during mammalian aging. On the other hand, epidemiological studies have shown correlations among the nutritional condition, increased plasma homocysteine (Hcy) and HL. Work with mouse models have further confirmed these correlations and suggested that nutritional supplementation may be beneficial for ARHL, but information is scarce on the associated molecular mechanisms.

We have used electrophysiological techniques (auditory evoked potentials), histology and immunohistochemistry, Western blotting and RT-qPCR, among other techniques. We present results on mouse models showing that: 1) IGF-1 circulating levels decrease with ageing showing a significant correlation with HL; 2) Low IGF-1 levels predispose to otic injury; 3) The lifelong cochlear gene expression profile of IGF system, cytokines and oxidation genes point to redox unbalance and inflammation as progression factors; 4) Methionine cycle genes are also regulated along ageing in the cochlea; and 5) The activity of stress kinases is regulated by age, injury and IGF-1. Our results strongly suggest that IGF-1, inflammatory cytokines and cochlear Hcy metabolism are determining factors for the onset and progression of ARHL.

S66.5
SPHINGOSINE 1 PHOSPHATE SIGNALING MODULATES OTIC NEUROGENESIS AND NEURODEGENERATION

Francesca Cencetti
Dipartimento di Scienze Biomediche Sperimentali e Cliniche "Mario Serio" Università di Firenze, Italy

Hearing loss represents one of the most prevalent chronic condition affecting older adults, that is dependent on the degeneration of hair cells and spiral ganglion neurons, which do not regenerate in mammals. Sphingosine 1-phosphate (S1P) is a bioactive sphingolipid capable of regulating many critical biological processes, such as cell proliferation, survival, migration and differentiation. S1P is produced by the ATP-dependent phosphorylation of sphingosine brought about by sphingosine kinase; after being exported outside through a specific transporter named Spns2, S1P exerts most of its actions by binding to five specific G protein-coupled receptors, S1P1-5. S1P signaling was recently shown to be involved in the maintenance of the auditory epithelium in postnatal mice. Indeed, S1P2 knock out mice display a profound deafness with loss of hair cells soon after birth and an alteration of stria vascularis. Similar effects are reported in Spns2 knock out mice, accompanied by a robust drop in the endocochlear potential. Nonetheless, many questions remain unanswered regarding the function of this sphingolipid in the inner ear.

Mouse otocyst-derived cell line was used as experimental model. These neuroblasts have an unlimited self-renewal ability while retaining their potential to differentiate into auditory neurons. Data will be presented regarding the role of S1P pathway in cell proliferation and survival as well as during the differentiation of neuroblasts to spiral ganglion neurons. Our results show that the biological effect of FGF2, which play important roles throughout inner ear development, is mediated by S1P signaling axis. The present study will contribute to clarify the molecular mechanisms that regulate S1P metabolism and S1P receptor expression in the various aspects of inner ear sensory cell biology, hopefully highlighting the therapeutic potential of S1P signaling pathway in hearing loss.

S67 Room: Reading Room
LANGUAGE LEARNING AND LEARNING DISORDERS

Chair: Mireille Besson, FR

S67.1
MUSIC TRAINING AND WORD

LEARNING

Mireille Besson
Laboratoire de Neurosciences Cognitives; CNRS & Aix-Marseille University, France

There is clear evidence in the literature that music training is associated with enhanced auditory perception of both musical sounds and speech sounds. Moreover, musicians typically show improved auditory attention and auditory short-term memory compared to non-musicians (e.g., George & Coch, 2011; Strait et al., 2010, 2015; Ho et al., 2003). This series of experiments aimed at going one step beyond, to test the hypothesis that music training also positively influences novel word learning, a task based on sound perception, attention, associative learning and memory. We used both behavioral and electrophysiological measures (ERPs) in both adults and children, with and without music training. In these two independent samples of participants, results showed that the percentage of correct responses in a semantic task, as well as the N400 effect (N400 to unrelated – N400 to related words), were positively enhanced by music training. Thus, these results provide strong evidence that the influence of music training goes beyond auditory perception and can facilitate the cognitive processes involved in learning the meaning of novel words. These findings will be discussed in terms of two complementary hypothesis (the cascade and multidimensional aspects of music training) and their impact for foreign language learning will be considered. Finally, the potential impact of our procedure to determine the origin of learning deficits in various patient populations (e.g., dyslexic children, Alzheimer patients. . .) will be underlined.

S67.2
UNDERSTANDING DEVELOPMENTAL DYSLEXIA: FROM CAUSES TO INTERVENTIONS

Johannes Ziegler
University Aix-Marseille and CNRS, France

Learning to read in alphabetic languages relies on two core mechanisms: phonological decoding and self-teaching. In this talk, I will present the first full-blown developmentally plausible computational model of reading acquisition that implements these two mechanisms. The model was used to simulate developmental trajectories of 622 children (388 dyslexics). I will show that individual reading performance on words and nonwords can be simulated with high accuracy on the basis of their underlying deficits in subcomponents of the reading network. Such simulations make it possible to predict for any given child how remediating one or several subcomponents should improve reading of words and nonwords. I will further show that common single-deficit theories are unable to account for the observed heterogeneity in reading performance. I thus advocate a multi-factorial computational approach of understanding reading and dyslexia, which has concrete practical implications for intervention.

S67.3
LANGUAGE AND EARLY INTERACTIONS

Benjelloun Ghizlane
CHU Ibn Rochd, Casablanca, Maroc Faculte de medicine Hassan II Laboratoire de neurosciences centre etudes doctorales, Morocco

Development, and therefore mental health and disturbance, takes place within the context of the care – giving relationships children have with their parents. Early care has a decisive, lifelong influence on how children learn, form relationships, experience and regulate their emotions and their behaviour. The majority of parents, the ordinary devoted mother/s and fathers, provide their children with a loving, safe home. 'Good enough' parents protect and comfort their babies, play with, praise and enjoy them. They also sometimes 'get it wrong' but try to rectify this in relation to the baby. Their babies respond, initiate, interact, explore and show pleasure. However, some parents find it very difficult to provide their children with conditions for this rounded development. In more extreme circumstances of hostile, neglectful environments, babies fail to thrive and their normal development is severely compromised or interrupted. There is mounting evidence for the severe long – term effects of disturbed early relationships and those characterised by neglect and abuse. Recent neuroscientific research has shown that brain development is affected as neuronal pathways 'fire' in response to the baby's experiences of being cared for. For instance, babies and young children who are repeatedly exposed to violence and other trauma, such that they feel severe and unmitigated distress over sustained periods, tend to become chronically anxious. This results from emotional experiences being 'hard wired' into the developing brain so that states of distress become characteristic features of the person's disposition or ways of being. Where the infant is at risk, early intervention can prevent later disturbance. Once a child's development has been derailed, it is important to intervene before maladaptive patterns of relating become set, since later intervention can only modify an already existing situation. Thus, early intervention will lead to more adaptive development and less disturbance in the first place

S67.4
DEVELOPMENTAL DYSLEXIA IN ARABIC-SPEAKING CHILDREN: UNIVERSAL FACTORS AND ORTHOGRAPHIC FEATURES

Smail Layes
Laboratoire de Psychologie et Neurosciences Investigations Cognitives du Neurone à la Société (ICONES) Normandie Université, Rouen, France

Arabic language has highly inflectional morphology and is characterized by specific vowellization system, making reading greatly demanding in terms of phonological and morphological processing. Two studies were conducted in order to examine the predictive relationships between word reading and reading comprehension as predicted variables, and the metalinguistic (phonological and morphological processing) as predicting factors. Results showed that phonological, speed of processing (RAN), and morphological awareness are deficient in Arabic-speaking dyslexic children and represent the basic predictors of dyslexia. These results are in line with the universal character of metalinguistic factors and show that reading in different languages is based on common cognitive processes. These results are discussed in terms of the impact of orthographic specificities and the universals of dyslexia.

S67.5
WORD PROCESSING IN ARABIC AND THE DIGLOSSIA QUESTION

Khateb Asaid
Unit for the Study of Arabic Language, Edmond J. Safra Brain Research Center for the Study of Learning Disabilities, Faculty of Education, University of Haifa, Haifa, Israel

Diglossia is considered as one of the most distinctive features of the Arabic language. Diglossia refers to a socio-linguistic situation in which two varieties of the same language are used for socially distinct functions. In the Arabic language, the Spoken Arabic (SA), acquired first, is used for everyday communications and conversation while literary Arabic (LA) is the language acquired at school for reading and writing and is used formal functions (writing, speeches and sermons). Since SA and LA differ in many of the linguistic aspects, and based on the history of acquisition and patterns of use of these two varieties, authors tend to consider SA as a first language (L1) and LA as a second language (L2) in literate Arabic speakers. However, the question of how these two varieties of Arabic are processed in the brain have not yet been understood.

In a series of studies using event-related potential (ER) and functional magnetic resonance (fMRI) techniques, we sought to provide insights into the neural basis of diglossia in Arabic. Electrophysiological and hemodynamic brain responses were analyzed in different experiments where healthy young subjects performed lexical decision and semantic decision task using SA and LA (and Hebrew as a control) words. For instance, in one experiment conducted in the visual modality, reaction time analysis showed that LA words induced the fastest responses and ERP analysis showed a modulation of brain responses during the early components. In another experiment in the auditory modality, SA induced faster responses than LA (and Hebrew) and ERPs showed a different response to SA in the 350 ms time range. Both RTs and ERPs reflected the ease with which each language variety was processed. The findings suggested that, when the issue is to process single words, the status of SA and LA as L1 and L2 is modality dependant: with SA showing dominance in auditory modality while LA showing dominance in the visual modality. Together with other observations, we suggest that diglossia can't be considered from one angle only, because the question of dominance becomes even more complicated during language production and lexical retrieval.

S67.6
LANGUAGE LEARNING: INSIGHT FROM THE NEUROPHYSIOLOGY OF SOCIAL LEARNING

Driss Boussaoud
Aix Marseille Univ, Inserm, INS, Institut de Neurosciences des Systèmes, Marseille, France

The emergence of social neurosciences has created an immense interest in the brain mechanisms of social modulation of behaviour. Yet, little attention has been devoted to the basic aspect, most fundamental aspect of social life, i.e. mere presence of others. Evidence accumulated for more than a century in social psychology demonstrates that the mere presence of conspecifics may improve performance on easy or well-mastered tasks (social facilitation), or impair performance on difficult or new tasks (social impairment). Two theories have been put forward to account for these phenomena: one holds that social facilitation increases the drive/arousal, the other suggests that mere presence of others affects attention focus. How does social presence modulate brain processes? It will be argued that this modulation may not be mediated by specialized brain areas or networks, as the concept of social brain suggest. Rather, as mere presence affects most behaviours ranging from perception to high level cognition, it may act on specialized neurons and networks. For example, a recent study found that learning-related neurons in the prefrontal cortex of monkeys are highly sensitive to social context. Some neurons code error-related signals preferentially under social presence (social neurons), whereas others are preferentially active under social isolation (Asocial neurons). Overall, learning performance appears to result from a dynamic interplay between these outcome-related neuronal populations. Thus, social context modulates the underlying neuronal processes, even on non-social as-

sociative tasks, calling for a re-thinking of the concept of the social brain. On a more practical front, this new evidence calls for caution when evaluating cognitive and affective abilities, particularly in children, where the stress level may drastically affect performance scores, and for the development of rehabilitation procedures that take into account the effects of social presence.

Minisymposia

MS1

MS1.1
ADENOSINE PLAYS A KEY ROLE IN CONTROLLING CA^{2+} FREE SEIZURES

Mark J. Wall
School of Life Sciences and Department of Mathematics, University of Warwick, UK

The complete removal or a reduction in the concentration of extracellular calcium ions (Ca^{2+}) can produce a paradoxical form of epileptiform activity in the hippocampus both *in vitro* and *in vivo*. Following Ca^{2+} removal CA1 pyramidal cells enter a state in which the membrane potential sits close to resting values with frequent short bursts of action potentials superimposed on small depolarisations. Occasionally, large depolarisations also occur that can either be spatially localised or can spread between neurons. This Ca^{2+}-free activity induced an increase in the extracellular concentration of adenosine, which activates adenosine A_1 receptors leading to hyperpolarisation of the membrane potential and the moderation of firing. During established Ca^{2+}-free activity, spontaneous adenosine–release events were superimposed on a tonic elevated concentration of extracellular adenosine. The major mechanism for both of these increases in extracellular adenosine was the movement of adenosine out of cells via equilibrative nucleoside transporters. Block of these transporters prevents the increase in adenosine concentration leading to hyper-excitation.

MS1.2
MODULATION OF INTRACELLULAR CHLORIDE TO REGULATE SYNAPTIC PLASTICITY AND RESCUE COGNITIVE FUNCTIONS IN DOWN SYNDROME

Gabriele Deidda
Neuroscience and Brain Technologies, Istituto Italiano di Tecnologia (IIT), Genova, Italy; Neuroscience Division, School of Bioscience, Cardiff University, UK; Department of Physiology and Biochemistry, University of Malta, Malta

Down syndrome (DS) is the most frequent genetic cause of mental retardation, with DS patients displaying low intelligence quotient, learning and memory impairment particularly in hippocampus-related functions. Trisomic mouse models of DS reproduce the main cognitive disabilities of the human syndrome. In particular, Ts65Dn mice show impaired synaptic plasticity (i.e., long-term potentiation, LTP) as well as learning and memory deficits. Increased GABAergic transmission through Cl-permeable $GABA_A$ receptors ($GABA_AR$) largely determines these deficits in DS mice. Indeed, LTP and cognitive impairment can be rescued reducing the magnitude of GABA-mediated signaling by treatment with GABAAR antagonists. Nevertheless, the efficacy of GABAergic transmission has never been directly assessed in DS. In this presentation, we will show that GABAergic signaling is excitatory rather than inhibitory and the reversal potential for $GABA_A$-driven Cl- currents (ECl) is less negative in hippocampi from adult DS mice. Accordingly, expression of cation/Cl- importer NKCC1 is increased in the hippocampus of trisomic mice and DS patients. Notably, NKCC1 inhibition by the FDA-approved drug bumetanide restores ECl, synaptic plasticity and hippocampus-dependent learning and memory in DS mice. Bumetanide recovers defects by means of acute rather than chronic effects. Our findings demonstrate that GABA is excitatory in DS adult mice, and identify a new and safe therapeutic approach to rescue cognitive disabilities of DS patients.

MS1.3
MATERNAL IMMUNE ACTIVATION AND ABNORMAL BRAIN DEVELOPMENT: NEW INSIGHTS ON NEURODEVELOPMENTAL DISORDERS PATHOGENESIS

Sara Anna Bonini
Department of Molecular and Translational Medicine, University of Brescia, Brescia, Italy

Aberrant immune activity during vulnerable and critical periods of neurodevelopment could participate in the generation of neurological dysfunction characteristic of several neurodevelopmental disorders (NDDs). Numerous epidemiological studies in humans have shown a link between maternal infections and NDDs risk; similarly, experimental studies using animal models of Maternal Immune Activation (MIA) support this association. One of the pathways with a main role in both neuron plasticity and immune system regulation is nuclear factor-κB (NF-κB). We have previously found that transgenic mice lacking the NF-κB p50 subunit ($p50^{-/-}$ mice) present cortical structural alterations and behavioural deficits.

In this study, we used an experimental and validated MIA-model, i.e. offsprings from LPS-treated mothers (LPS-mice), and a genetic model of immune dysfunction, i.e. $p50^{-/-}$ mice, to investigate on the role of NF-κB pathway in the pathophysiology of NDDs. Offsprings from $p50^{-/-}$, LPS- and wild-type mice were analyzed for inflammation markers, cortical structure and behavioral abnormalities.

In line with previous data, we found that $p50^{-/-}$ and

LPS-mice display a chronic inflammation phenotype. Indeed, we found an increased brain gliosis and altered cytokines serum levels. When compared to wild-type mice, both $p50^{-/-}$ and LPS-mice at post-natal day 2 present altered BLBP (radial glial cells marker) and Reelin protein expression levels, in addition to specific alterations in cortical layering. Moreover, adult $p50^{-/-}$ mice display abnormal columnar organization in the somatosensory cortex, altered neurite orientation and decreased Synapsin I protein levels. Finally, both $p50^{-/-}$ and LPS-mice present increased locomotor and exploratory activity, reduced social interaction, and impaired communication. Taking together, these data provide new insight on the possible link between altered immune function, NF-κB pathway and pathogenesis of NDDs.

MS2

MS2.1
STUDY OF ARC-MEDIATED CHANGES IN THE MORPHOLOGY OF DENDRITIC SPINES DURING AMNESIA AND ITS RESTORATION

Akash Gautam
Centre for Neural and Cognitive Sciences, University of Hyderabad, Hyderabad, India

Our previous study shows that both amnesia (induced by scopolamine) as well as its restoration (with the pre-treatment of i-Extract i.e. alcoholic leaf-extract of *Withania somnifera*) modulates the expression of neuronal immediate early gene- Arc in the brain of Swiss albino adult male mice. Since i-Extract attenuates the scopolamine-induced amnesia by upregulating the expression of Arc mRNA and protein, we hypothesized that Arc is involved during the process of amnesia and its restoration.

Therefore, we examined the function of Arc i.e. actin polymerization and subsequent changes in the dendritic spines, by specific inhibition of its transcription and translation in the hippocampus of mouse.

We observed that stereotaxic infusion of Arc antisense oligodeoxynucleotides (ODN) in CA1 region decreased the level of Arc protein which was attenuated when treated with i-Extract prior to infusion of Arc antisense ODN. We noted a significant decrease in the polymerization of F-actin during scopolamine-induced amnesia as well as Arc antisense ODN infusion that was restored rather enhanced when pre-treated with i-Extract in both the cases. We also compared the changes between CA1 (the infusion site) and CA3 (neighbouring site of infusion) regions of hippocampus, and found more pronounced effects in CA1 than in CA3 region. The extent of F-actin polymerization, as revealed by changes in the dendritic spine architecture through Golgi staining, showed that both scopolamine as well as Arc antisense ODN disrupted the spine density and mushroom shaped morphology that was again regained if pre-treated with i-Extract.

In conclusion, the findings reveal that Arc helps in polymerization of F-actin and subsequent changes in the morphology of dendritic spines after pre-treatment with i-Extract in scopolamine-induced amnesic mice, suggesting a definite role of Arc during scopolamine-induced amnesia and its recovery by i-Extract.

MS2.2
DEEP BRAIN STIMULATION AS A NEW SERVICE IN MALTA THROUGH CROSS BORDER COLLABORATION: AUDIT OF THE FIRST FIVE YEARS

Josanne Aquilina
Mater Dei Hospital, Tal-Qroqq, Msida, Malta

Background: Deep Brain Stimulation (DBS), a well-established treatment for Parkinson's Disease (PD) with motor fluctuations on medical therapy, has been introduced as a new service in Malta through a cross border collaboration between specialist services in London and a tertiary centre in Malta.

Objectives: The number of patients in Malta likely to benefit from DBS was envisaged to be between 5 to 10 per year. The purpose of this study was to determine the success of this international collaboration.

Methods: Between 2011 and 2015, the total number of patients undergoing deep brain stimulation was 35. Of these, 29 patients received bilateral Subthalamic Nucleus (STN) DBS for Parkinson's Disease. Pre-operative motor function was compared with one year post-operative motor function assessments in 26 patients. Pre-operative and post-operative quality of life assessment was also completed in 24 patients.

Results: There was a statistically significant improvement in off-medication Unified Parkinson's Disease Rating Scale (UPDRS) III motor function (41.7%), reduction in Levodopa Equivalent Dose (LED) (30.6%) and improvement in quality of life as measured by the Parkinson's Disease Questionnaire (PDQ-39) (52.3%). Sub analysis of the PDQ-39 dimensions showed significant improvement in all dimensions except communication, with greatest benefit for activities of daily living (ADLs) (72.4%) and stigma (66.3%). Surgical adverse events were transient with no permanent sequelae. Patients receiving DBS to targets other than the STN and for different indications are also described.

Conclusion: This audit demonstrates the success of delivery of specialist services through cross border collaboration with achievement of expected results in terms of therapeutic benefit to patients.

MS2.3
INVOLVEMENT OF THE LYMPHATIC AND GLYMPHATIC SYSTEMS IN ALZHEIMER'S DISEASE

Goran Šimić
Department of Neuroscience, Croatian Institute for Brain Research, School of Medicine, University of Zagreb. Šalata 12, 10 000 Zagreb, Croatia

While it is generally considered that stroke occurs only in the context of vascular disease, recent evidence shows that all underlying processes leading to stroke depend on interactions of neurons, glial cells, vascular endothelial cells and pericytes, and components of the extracellular matrix, altogether referred to as a neurovascular unit, and that recovery after stroke also depends on the interaction of all structures within the neurovascular unit. Vascular disease and Alzheimer's disease (AD) have long been considered specific disease entities, mainly because the neuropathological changes that characterize AD are the deposition of amyloid β peptide (Aβ) in the brain parenchyma (amyloid or senile plaques, SP) and Aβ accumulation in the walls of cerebral arteries, arterioles, capillaries and veins (cerebral amyloid angiopathy, CAA), dysfunctions of the blood-brain barrier and the accumulation of neurofibrillary tangles (NFT) in neurons, whereas cerebrovascular disease can lead to vascular cognitive impairment (VCI) in different ways than AD. Most commonly, the underlying mechanism of stroke is ischemic (usually thrombotic, sometimes embolic, rarely due to systemic hypoperfusion), and in about 15% of cases it is hemorrhagic (most often as intracerebral hemorrhage, and sometimes as subarachnoid hemorrhage). The currently prevailing opinion is that although AD and VCI can exist as separate entities, both conditions co-occur in as many as 60% of all patients with cognitive decline (Querfurth and Lafer, 2010). In one study, of all patients with a syndrome of dementia, 38% fulfilled the criteria for both AD and VaD, 30% had AD, 12% VaD, and the remaining 10% simultaneously met the criteria for AD and Lewy body disease or Parkinson's disease and AD (Schneider et al., 2007). The prevalence of CAA in patients with AD varies from 70–100% (Tian et al., 2004), but more important is the fact that CAA in large blood vessels is characterized by the predominant deposition of $A\beta_{1-40}$ and is usually not associated with AD but with VCI, while the disposal of the longer form ($A\beta_{1-42}$) in pericapillary spaces (CapCAA) is highly associated with AD (Attems et al., 2004). These observations also support the claim that the main source of Aβ is neurons, Aβ being further drained through the interstitial fluid and deposited along the basement membrane of brain capillaries and larger blood vessels. It is well known that the longer form of the peptide ($A\beta_{1-42}$) is strongly related to abnormal aggregation than the shorter form ($A\beta_{1-40}$), and therefore its accumulation is considered a higher risk for the development of AD. I propose that a strong mechanism must exist in cerebral blood vessels that opposes clot formation as is the case in the periphery, to prevent stroke. Thus, prevention of microbleeds by Aβ might represent an evolutionary adaptation of the amyloid precursor protein (APP) anticoagulant function in the central nervous system to serve as a stroke-preventing agent. This is supported by the fact that APP is a soluble isoform of protease inhibitor nexin-2 (PN-2), as both of these proteins strongly inhibit coagulation factor XIa in peripheral blood, and by the fact that two out of five mutations in exons 16 and 17 of the APP gene, which encode part of the APP molecule from which later proteolytic cleavage by β- and γ-secretase will produce Aβ, cause fatal stroke in adulthood: the Flemish mutation (Cys692Gly) and the Italian mutation (Glu693Lys); however, two other mutations in the same region of the APP gene, are relatively less frequent and cause early AD: the Arctic mutation (Glu693Gly) and the Osaka mutation (Glu693Asp) (for review, see Šimić et al., 2017). By secretion from vascular endothelial cells in the brain and from neurons, PN-2 and Aβ could serve as preventative agents against stroke. As there is apparently no feedback signal or signalling pathway through which neurons receive the stop signal to cease creating Aβ by cleaving APP using the amyloidogenic pathway, neurons continue in AD to generate additional amounts of Aβ. Over time, the excess of Aβ precipitates and turns into insoluble aggregates, which further oligomerize and aggregate into insoluble fibrils in the form extracellular SP. In AD, this process is likely induced by reduced outflow of Aβ through the glymphatic system, which serves to clear molecules accumulating in the interstitial fluid. Recent data suggest that this process is principally dependent on the influx of water through aquaporin (AQP4) channels on astroglial processes that contribute to the blood-brain barrier. Other genetic, epigenetic, and microenvironmental factors are also involved. One of them could be leakage of divalent ions from plasma through a deficient blood-brain barrier into the neuropil. Divalent ions have been shown in vitro to accelerate the aggregation of Aβ. Until recently the central nervous system was considered to lack a system of lymphatic vessels that drains interstitial fluid and allows circulation of immunocompetent cells, but this view changed when lymphatic vessels where described in the mouse brain (Louveau et al., 2015). Most of these lymphatic vessels are aligned along the transverse and the upper sagittal sinuses, and contain cerebrospinal fluid (CSF), T lymphocytes, and dendritic cells. It was also demonstrated that these meningeal lymphatic vessels drain into the deep lymph nodes of the

neck using Evan's Blue (Louveau et al., 2015). Therefore, dysregulation or disruption of the blood-brain barrier and the glymphatic and lymphatic systems harbors the potential to contribute to the development of neurodegenerative diseases, particularly AD, and represent a potentially important and accessible target for the treatment of AD, and other neurodegenerative conditions.

MS3

MS3.1
EFFECTS OF CORTICOSTERONE ON THE INITIATION OF 50 kHz ULTRASONIC VOCALIZATIONS AND ON CALLING STIMULATED BY SOCIAL CONTACTS AND AMPHETAMINE

Nicola Simola
Department of Biomedical Sciences, Section of Neuropsychopharmacology, University of Cagliari, Cagliari, Italy

Rats emit 50 kHz ultrasonic vocalizations (USVs) in appetitive situations and these USVs are increasingly being used as a tool in preclinical studies on reward and motivation. Although the emission of 50 kHz USVs is initiated by the activation of dopamine receptors, this behavior can be modulated by non-dopaminergic receptors and recent evidences suggest that changes in the levels of corticosterone might influence calling in the 50 kHz frequency range.

In order to elucidate the interplay between corticosterone-mediated signaling and 50 kHz USVs, this study evaluated the effects of corticosterone (1–5 mg/kg, s.c.), of the corticosterone synthesis inhibitor metyrapone (50 or 100 mg/kg, i.p.), and of the glucocorticoid receptor antagonist mifepristone (40 or 100 mg/kg, s.c.) on the initiation of 50 kHz USVs and on calling during non-aggressive social interactions and after the administration of amphetamine (1 mg/kg, i.p.), two situations that possess positive affective valence.

Administration of corticosterone failed to initiate the emission of 50 kHz USVs and did not affect calling during social interactions and after amphetamine administration. Similarly, metyrapone and mifepristone did not to initiate the emission of 50 kHz USVs. However, metyrapone, suppressed calling during social interactions and after amphetamine administration. These effects of metyrapone were not reproduced by mifepristone and by the mineralocorticoid antagonist spironolactone (50 or 75 mg/kg, s.c.), which had no effects on calling.

Taken together, these results demonstrate that corticosterone does not influence the emission of 50 kHz USVs and suggest that metyrapone suppresses calling in the 50 kHz frequency range by mechanisms different from the inhibition of steroidogenesis.

MS3.2
SYNERGISTIC INTERACTIONS BETWEEN OPIOID AND CANNABINOID SYSTEMS IN OBESITY

Claudio D'Addario
University of Teramo, Biosciences, Teramo, Italy; Karolinska Institutet, Clinical Neuroscience, Stockholm, Sweden

Obesity is an urgent public health problem, potentially affecting emotional and physical health. Core symptoms of this disease are disturbance of eating habits and inability to control body weight, thus with an imbalance between energy intake and expenditure. It is now clear that rewarding properties of food are responsible for obesity development. Among the endogenous signaling networks involved in both rewarding and homeostatic mechanisms, a relevant role is played by the endocannabinoid (ECS) and by the opioid (EOS) systems, which functionally interact with each other.

We used the Diet-induced obesity (DIO) rat model to analyze the epigenetic regulation of ECS and EOS genes expression in rats placed on a high-fat diet (HFD). Moreover, in a subset of obese human subjects and healthy controls, we assessed DNA methylation levels at target gene promoters, and also genotyped Single nucleotide polymorphisms (SNPs) within cannabinoid receptor type 1 (CB_1) and mu opioid receptor (MOP) genes already associated with obesity.

After 5 weeks of HFD, gene expression analysis in the hypothalamus revealed a selective and significant increase of CB1 and MOP mRNA levels in DIO rats. Instead, no changes were observed in rats maintained under the diet regimen for up to 21 weeks for any other ECS and EOS genes. Consistently, epigenetic studies showed a selective and significant decrease in DNA methylation at specific CpG sites of CB_1 and MOP gene promoters in DIO rats. Moreover, after data stratification in human obese subjects, we observed significant changes in DNA methylation levels at CB_1 promoter in younger subjects and in those reporting eating disorders.

Taken together, we here provide evidence of selective, synergistic and time-dependent transcriptional regulation of CB_1 and MOP genes in DIO rats, as well as (at least to a certain extent) in human subjects. The alterations in gene regulation would contribute to develop overweight in DIO rats, possibly due to the hedonic impact of palatable food in these animals. Studying how environmental factors might induce obesity would be of help to understand which changes occur in central circuits, and to provide new insights for possible interventions, through either nutrition or specific drugs, aimed at modifying obesity risk.

MS3.3
EFFECTS OF NICOTINE ON COGNITIVE FUNCTION ACROSS THE MENSTRUAL CYCLE IN NON-SMOKING WOMEN

Adrianna Mendrek
Department of Psychology, Bishop's University, Quebec, Canada

While the neurocognitive effects of nicotine and estradiol have been well documented, the interaction between the two remains largely unexplored. The acute administration of nicotine improves attention and working memory (WM) in abstinent smokers, but the effects on non-smokers are less clear. In women, elevated levels of estrogen during the late follicular and mid-luteal phase of their menstrual cycle (MS) have been linked to improved performance on WM and emotion processing, while low levels during early follicular phase, have been associated with enhanced viso-spatial abilities. The role of progesterone (another hormone fluctuating across MS) is less well understood; however, its metabolite - allopregnanolone has high affinity for $GABA_A$ receptors and increased levels of GABA have been linked to improved performance on WM. In the present study, we investigated the effects of nicotine on cognition across the MC in healthy non-smoking women. Participants were tested with 4 mg nicotine gum and with a placebo gum during three phases of their MC: early follicular, late follicular and mid luteal, in a randomized manner, resulting in six tests per participant. Participants completed four cognitive tests on each occasion: WM, verbal memory, emotion identification, and visuo-spatial processing. Preliminary results suggest that administration of nicotine leads to the overall diminished performance on the WM across MC, while other cognitive domains remain unaffected. WM function was best during the mid-luteal phase in the placebo condition, but nicotine interfered with it to the greatest extent during this time. Nicotine's deteriorating effect on WM in non-smoking women is inconsistent with previous results in men. We are in the process of collecting more data and updated findings will be presented during the conference.

MS4

MS4.1
EFFECT OF GLYPHOASTE SUBCHRONIC AND CHRONIC EXPOSURE IN JUVENILE MICE: BEHAVIORAL AND IMMUNOHISTOLOGICAL STUDY

Yassine Ait Bali
Laboratory of Pharmacology, Neurobiology and Behavior (URAC 37), Cadi Ayyad University, Marrakech, Morocco

Many epidemiological studies have described an adolescent-related psychiatric illness, and sensorimotor deficits after Glyphosate based herbicide (GBH) exposure, and reports of GBH exposure in animal models suggest that it may be neurotoxic and could impact brain development and behavior in adulthood. However, its neurotoxic effects on neonatal, immature brains remain unclear and the results are conflicting.

The present study was conducted to evaluate the postnatal GBH following acute, subchronic (6 weeks) and chronic (12 weeks) exposure (250 or 500 mg/kg/day) on behaviors related to anxiety and depression-like, and exploratory activity in juvenile (prepubertal) mice. Animals were tested using behavioral paradigms as the open field, the elevated plus maze, the tail suspension and splash test. After completion of the behavioral testing, adult mice were sacrificed and the expression of tyrosine hydroxylase (TH) in the substantia nigra pars compacta (SNc) and serotonin in the dorsal raphe nucleus (DRN), the basolateral amygdala (BLA) and the ventral medial prefrontal cortex (mPFC) was evaluated using immunohistochemical procedure.

Our results indicate that unlike acute exposure, both subchronic and chronic exposure to GBH induced a decrease in body weight gain and locomotor activity, and an increase of anxiety and depression-like behavior levels. In addition, the immunohistochemical findings showed that the only chronic treatment induced a reduction of TH-immunoreactivity in the sink. However, both subchronic and chronic exposure produced a reduction of serotonin-immunoreactivity in the DRN, BLA and ventral mPFC.

Taken together, our data suggest that exposure to Glyphosate based-herbicide leads to neurobehavioral changes that stem from the impairment of neuronal developmental processes in juvenile exposed mice.

MS4.2
THE RELATIONSHIP OF PERSONALITY, SPIRITUALITY AND POSTTRAUMATIC GROWTH TO SUBJECTIVE WELLBEING

Michael Galea
University of Malta, Msida, Malta

A growing number of studies are indicating that a number of people report psychological growth after experiencing trauma. This may be so because suffering stimulates the need and search for meaning [1]. In this cross-sectional and correlational study, we sought the relationship of subjective well-being to posttraumatic growth in view of past trauma experiences and perceived stress. In particular, we investigated a sample of tertiary students' perceived stress, past traumas, subjective well-

being, faith maturity, positive and negative affect, and personality, together with demographic correlates. Past traumas included loss of a loved one, chronic or acute illness, injury, divorce, violent crime, and job loss, amongst others. Only a quarter of respondents experienced their trauma/s 5 years or more prior the study, thus indicating relatively recent trauma experiences. Post-traumatic growth correlated with personality, faith maturity, wellbeing and positive affect. In examining the patterns of correlations noted above, a hierarchical multiple regression analysis was employed. Posttraumatic growth was found to have unique variance even after partialling out key variables such as perceived stress, personality and faith maturity. Although situational factors and personality did play important roles, this study clearly points at the relevance of faith maturity and posttraumatic growth for the promotion of holistic wellbeing of those affected by trauma. Religious beliefs may counter hopelessness and form an important buffer in this equation. The psycho-social implications of these results were discussed.

MS4.3
SEX DIFFERENCES IN EXPERIMENTAL STUDIES OF DEPRESSION: HOW CAN CLINICAL RESEARCH BENEFIT?

Nikolaos Kokras
Department of Pharmacology, Medical School, National & Kapodistrian University of Athens, Greece; First Department of Psychiatry, Eginition Hospital, Medical School, National & Kapodistrian University of Athens, Greece

Sex differences in depression and antidepressant response in humans are modestly studied and results are controversial. Experimental studies using animal models may provide insights that could be useful in clinical trials. We summarize findings from preclinical studies on sex differences in the phenotype of depression, its endophenotype and the antidepressant response and suggest how such preclinical research might be of use in clinical research [1].

In preclinical studies behavioral indices and models are adjusted for both sexes, in order to properly identify sex differences in primary outcomes. This is not routinely happening in clinical studies when using depression rating scales, which is the analogue of behavioural indices. Moreover, preclinical studies show sex differences at the baseline behavioral response and underlying mechanisms that often converge following antidepressant treatment. This is also a neglected issue in human studies. Finally, preclinical research suggests that when researching on potential biomarkers for depression and antidepressant response sex should be an important factor to consider.

Cautious exploitation of findings on sex differences from preclinical research could improve the design and quality of clinical studies for disease biomarkers and novel antidepressants and facilitate the drug development in a gender aware manner [2].

[1] Kokras N & Dalla C. (2014). Sex differences in animal models of psychiatric disorders. *Br J Pharmacol.*, *171*(20), 4595–4619. [2] Kokras N & Dalla C. (2017). Preclinical sex differences in depression and antidepressant response: Implications for clinical research. *J Neurosci Res.*, *95*(1–2), 731–736.

MS5

MS5.1
DOPAMINE METABOLISM ANALYSIS IN DIFFERENT CELLULAR MODELS OF PARKINSON'S DISEASE

Carmen de la Fuente
Genetics and Genomic Medicine UCL GOS Institute of Child Health, London, UK

Dopamine (DA) is a neurotransmitter which loss leads to the symptoms of Parkinson's disease (PD). However, the underlying causes of dopaminergic degeneration in PD are unknown. Among the mechanisms proposed, mitochondrial and lysosomal impairment have been proposed as key players in the neuronal cell death associated with PD. Thus loss of mitochondrial complex I or lysosomal glucocerbrosidase (GBA1) have both been reported in PD brains. In order to evaluate further the potential role of complex I or GBA1 deficiency in PD, we have studied the effect of loss of such enzyme activity on dopamine metabolism.

The SH-SY5Y human neuroblastoma cell line was the in vitro model chosen. First, to model the mitochondrial impairment, the cells were treated with the complex I inhibitor rotenone. Second, to model of lysosomal dysfunction, we used conduritol B epoxide, a well-stablished GBA1 inhibitor. Finally, we treated the cells with L-DOPA, the dopamine precursor that is used as PD treatment. Dopamine and its metabolites were quantified in the extracellular media by high-performance liquid chromatography. Additionally, mRNA and protein expression and, activity of the enzymes involved in the pathway were studied.

Mitochondrial or lysosomal impairment resulted in a modified dopamine catabolism. The extracellular concentration of DOPAC, the immediate dopamine metabolite produced by MAO, was significantly increased after rotenone or CBE treatments by 245 and 587% respectively. In contrast, the same cells showed a decreased release of HVA, a dopamine metabolite requiring COMT activity. To ascertain whether these observations

were due to transcriptional, translational or functional changes, real time PCR, Western blot and activity assays are being carried out.

Our results suggest that the mitochondrial and lysosomal impairment observed in PD may have an effect on dopamine metabolism, enhancing dopamine depletion and increasing oxidative stress by MAO-derived hydrogen peroxide production. In conclusion, dopamine metabolism is modified when mitochondrial complex I or GBA1 are impaired, possibly due to changes on the expression or activity of the catabolic enzymes.

MS5.2
EXTRACTS FROM TWO UBIQUITOUS MEDITERRANEAN PLANTS AMELIORATE CELLULAR AND ANIMAL MODELS OF NEURODEGENERATIVE PROTEINOPATHIES

Michelle Briffa
Dept. of Physiology & Biochemistry, University of Malta, Msida, Malta

A signature feature of age-related neurodegenerative proteinopathies is the misfolding and aggregation of proteins, typically amyloid- β (Aβ) in Alzheimer's disease (AD) and α-synuclein (α-syn) in Parkinson's disease (PD), into soluble oligomeric structures that are highly neurotoxic. Cellular and animal models that faithfully replicate the hallmark features of these disorders are being increasing exploited to identify disease-modifying compounds. Natural compounds have been identified as a useful source of bioactive molecules with promising neuroprotective capabilities.

In the present report, we investigated whether extracts derived from two ubiquitous Mediterranean plants namely, the prickly pear Opuntia ficus-indica (EOFI) and the brown alga Padina pavonica (EPP) alleviate neurodegenerative phenotypes in yeast (Saccharomyces cerevisiae) and fly (Drosophila melanogaster) models of AD and PD.

Pre-treatment with EPP or EOFI in the culture medium significantly improved the viability of yeast expressing the Arctic Aβ42 (E22G) mutant. Supplementing food with EOFI or EPP dramatically ameliorated lifespan and behavioural signs of flies with brain-specific expression of wild-type Aβ42 (model of late-onset AD) or the Arctic Aβ42 variant (model of early-onset AD). Additionally, we show that either extract prolonged the survival of a PD fly model based on transgenic expression of the human α-syn A53T mutant.

Taken together, our findings suggest that the plant-derived extracts interfere with shared mechanisms of neurodegeneration in AD and PD. This notion is strengthened by evidence demonstrating that EOFI and to a greater extent EPP, while strongly inhibiting the fibrillogenesis of both Aβ42 and α-syn, accumulate remodelled oligomeric aggregates that are less effective at disrupting lipid membrane integrity. Our work therefore opens new avenues for developing therapeutic applications of these natural plant extracts in the treatment of amyloidogenic neurodegenerative disorders.

MS5.3
THE 6-HYDROXYDOPAMINE HEMIPARKINSONIAN RAT MODEL: EVIDENCE OF EARLY STAGE DEGENERATION OF THE NIGROSTRIATAL PATHWAY

Tiziana Marilena Florio
Department of Life, Health and Environmental Sciences, University of L'Aquila, L'Aquila, Italy; Laboratori Nazionali del Gran Sasso, Istituto Nazionale di Fisica Nucleare, L'Aquila, Italy

In human Parkinson Disease (PD) as well as PD rat models, during the early phase of degeneration spared neurons act through a number of compensatory mechanisms that are still not fully understood. Our aim was to characterise this early stage of the intranigral 6-OHDA lesion in rats.

Unilaterally 6-OHDA (8 µg/4 µL) and sham-injected rats underwent the Open Field (OF) test in two different behavioural paradigms: drug-free (10 min), and Apo-induced condition (0.5 mg/kg, 60 min). The Unrepeated Group (UG, $n = 18$) underwent a single Apo injection, the Repeated Group (RG, $n = 14$) received multiple treatments. Locomotor activity was collected, measuring contralateral turning rate in the OF arena, before lesion (day 0) and between 1–21 days. The rat brains were then analysed by means of *ex-vivo* high-resolution MRI and TH+ immunohistochemistry.

Our behavioural data revealed that the post-synaptic DA receptor sensitization developed within 7 days from DA injury. The repetitive treatment with the DA agonist, probably acting on the TH activity, seems to interfere with compensatory mechanisms soon after the injury, producing a hyper-sensitization, i.e. a faster turning just during the first week. *Ex-vivo* MRI analysis showed structural changes and alteration of the GM/WM properties in the ipsilateral striatum. TH immunostaining revealed a patchy-like distribution in the Striatum and a partial DA depletion of the nigrostriatal pathway within 7 days (early) as well as a near complete degeneration at 21 days (late).

In conclusion, we observed a clear correlation between apomorphine-induced motor responses and striatal tissue structural changes during the early stage of PD in rats. Hopefully, with the advent of ultra-high-field clinical MRI, this may be useful for the early diagnosis of the disease and, with the understanding of the associated biological mechanisms, give useful indications for a

timely therapeutic intervention.

MS6

MS6.1
TPH2-DEFICIENT RATS SHOW ALTERATIONS OF NEUROPLASTIC MECHANISMS IN BASAL CONDITION AND AFTER AN ACUTE STRESS

Francesca Calabrese
Department of Pharmacological and Biomolecular Sciences, University of Milan, Milano, Italy

It is well established that alterations of the serotonergic system may contribute to the pathophysiology of mood disorders. In this study, we used rats deficient in TPH2, the enzyme responsible for serotonin synthesis in the central nervous system. We took advantage of this animal model, lacking serotonin specifically in the brain, to investigate whether a vulnerable genotype can be associated with alterations of neuronal plasticity, which appear to be relevant for psychopathological risk. Moreover, exposing these rats to an acute stress we aimed to clarify the influence of serotonin on the response to a challenging situation.

We analyzed the expression of the Brain-Derived Neurotrophic Factor (BDNF), a neurotrophin, known to play an important role in adult neuronal plasticity and associated with stress response. Adult male and female TPH2-deficient ($TPH2^{-/-}$) and wildtype ($TPH2^{+/+}$) rats were exposed to an acute restraint stress and sacrificed at two different time points. Gene expression and protein levels of BDNF and of other plasticity markers were then analyzed in the prefrontal cortex and in the hippocampus.

We found an increase of all the forms of Bdnf measured, in the prefrontal cortex of $TPH2^{-/-}$ male rats, whereas in $TPH2^{-/-}$ females the upregulation was specific for the total and isoform IV mRNA levels.

Interestingly, the response to the stress exposure was influenced by the lack of serotonin in a gender-specific manner: in females the increase in total BDNF mRNA expression induced by the acute stress was 2 fold higher in $TPH2^{+/+}$ compared to $TPH2^{-/-}$, while in males we found a different temporal profile of changes in the induction of the long 3′UTR BDNF transcripts.

In summary, our results showed that serotonin deficiency not only affects neuroplastic mechanisms in basal condition, but mostly, influences the response to an acute stress suggesting an impairment in the coping strategies set in motion by a challenging condition.

MS6.2
ISATIN DERIVATIVE INHIBITS NEURONAL PATHOLOGY INDUCED BY REACTIVE COMPOUNDS PRESENT IN PROCESSED SUGARS

Meha Fatima Aftab
Dr Panjwani Center for Molecular Medicine and Drug Research, ICCBS, University of Karachi-75270, Karachi, Pakistan

Consumption of high processed sugars has become a part of contemporary culture. The processing of sugars lead to formation of highly reactive glycotoxins like methylglyoxal which has been shown to damage neuronal processes by inducing oxidative stress. Methylglyoxal also promotes insulin resistance by inhibiting Ser473 phosphorylation of AKT and Ser9 phosphorylation of GSK-3β, a mechanism proposed for neurodegeneration. The current treatment for neurodegenerative diseases increases acetylcholine levels however; neurodegeneration persists that worsens the clinical outcomes. Synthetic Schiff base of naturally occurring isatin has been shown to inhibit glycotoxins. The objective of this study was to evaluate mechanistic effect of isatin derivative for prevention of neurodegeneration in brain.

Insulin resistance in neuro2A cells was induced by methylglyoxal followed by treatment with isatin derivative. Phosphorylation of Ser 473 and Ser 9 of Akt and GSK-3β were analyzed respectively using western blotting. Effect of isatin derivative on axonal integrity was studied by differentiating Neuro2A using retinoic acid.

Isatin derivative lowered methylglyoxal induced stress in neuro2A, inhibited methylglyoxal mediated downregulation of insulin signaling via restoration of serine phosphorylation of Akt and GSK-3β and restored axonal length in differentiated neuro2A.

Our findings suggest that isatin derivative can develop as a new therapeutic agent for treatment of neurodegeneration.

MS6.3
EFFECTS OF DHA LOW IN PHYTANIC ACID (OPHY3) IN MEMORY AND BIOMARKERS OF NEURODEGENERATION IN HIPPOCAMPUS OF $APOE^{-/-}$ MICE FED A HIGH FAT DIET

María Belén
Department of Physiology, School of Medicine, Complutense University, Madrid, Spain

Docosahexaenoic acid (DHA) is a polyunsaturated omega-3 fatty acid. Previous studies demonstrated its neuroprotective properties, and could be an effective treatment for neurodegenerative diseases, like Alzheimer's Disease (AD). Despite the evidence of these benefits, some studies observed a reduced efficacy that could be due to its high content of phytanic acid (PA),

which could counteract the effects of DHA.

The aim of the study was to investigate the effect of DHA with low concentration of PA (OPHY3, 50 ppmPA) on cognition, emotion and spontaneous motor activity, as well as the hippocampal protein expression of the main biomarkers involved in AD. We used 27 ApoE$^{-/-}$ mice, fed a high fat diet and divided into three groups: 1) ApoE; 2) ApoE+OPHY3; 3) ApoE+DHA+1000 ppmAF. In addition, 27 C57BL/6 mice, fed a standard diet, were divided into 3 groups: 1) Control; 2) Control+OPHY3 3) Control+DHA+1000 ppmAF. DHA (10%) and PA were added in the diets (Natac Biotech, S.L.). After 42 days of treatment, spontaneous locomotor activity, anxiety levels and spatial memory were evaluated. At 70 days, hippocampal protein expression of interleukin-6 (IL-6), brain-derived neurotrophic factor (BDNF), tumor necrosis factor α (TNFα), inducible nitric oxide synthase (iNOS), Caspase 3 (Cas-3), β-amyloid precursor protein (APP) and β-amyloid peptide (Aβ) were evaluated by Western blot.

The three ApoE groups showed a significant decrease in spontaneous locomotor activity and higher levels of anxiety compared to the control groups. ApoE+OPHY3 group succeeded in reversing memory deficits, improving spatial memory and cognitive ability. Hippocampus protein expression of IL-6, TNF-α, iNOS, Cas-3, APP and Aβ decreased in ApoE+OPHY3 group, but increased expression of BDNF, versus ApoE and ApoE+DHA+1000 ppmAF groups.

In conclusion, these results suggest that DHA with low concentration of PA (OPHY3) could be an effective treatment in neurodegenerative diseases such as AD.

Posters

Monday, June 12th 2017

P12.1
ANTI-INFLAMMATORY DRUGS EXERT ANTIDEPRESSANT-LIKE EFFECTS AND REDUCE BRAIN LEVELS OF IL-6 IN RATS

Kaplanski J[1], Nassar A[1] and Azab AN[1,2]
[1]*Department of Clinical Biochemistry and Pharmacology*
[2]*Department of Nursing – Faculty of Health Sciences, Ben-Gurion University of the Negev; Beer-Sheva, 84105, Israel*

A large body of data suggests that inflammation plays a role in the pathophysiology and treatment of mood disorders. Consistently, anti-inflammatory drugs were found to exert mood-stabilizing effects in randomized clinical trials of mood disorders patients.

This study was undertaken to: i) Examine the antidepressive-like effects of the anti-inflammatory drugs dexamethasone (DXM, a potent anti-inflammatory corticosteroid) and pentoxifylline (PTF, a tumor necrosis factor-α inhibitor) in rats subjected to a depression-inducing protocol; and, ii) determine the effects of DXM and PTF on interleukin (IL)-6 levels in hypothalamus (HT) and hippocampus (HC) of the "depressed" rats.

Rats were subjected to an unpredictable chronic mild stress (UCMS) protocol for 6 week during the last 2 weeks of which they were treated (intraperitoneally) daily with DXM (1 or 2 mg/kg) or PTF (10 or 50 mg/kg). Rats were subjected to a sucrose consumption test at different time-points. Moreover, at the end of DXM and PTF treatment rats were subjected to a forced swim test (FST). One day after FST rats were sacrificed, their brains were ousted and HT and HC were extracted. HT and HC were homogenized, centrifuged and supernatants were separated for determination of IL-6 levels by ELISA.

Sucrose consumption was significantly lower in UCMS rats. PTF but not DXM significantly increased sucrose consumption in UCMS rats, suggesting an antidepressant-like effect of PTF. Consistently, PTF significantly reduced immobility time in the FST. Moreover, PTF significantly decreased IL-6 levels in HT and HC while DXM (2 mg) reduced IL-6 levels only in HC. These results suggest that PTF exhibits antidepressive-like effects which may be associated with its inhibitory effect on IL-6 production in the brain.

P12.2
NEURONAL AND BEHAVIORAL CORRELATES OF PHASIC AND SUSTAINED FEAR IN FREELY BEHAVING MICE

Seidenbecher T, Remmes J, Daldrup, T, Lesting J and Pape H-C
Institute of Physiology I, Westfälische Wilhelms-University Münster, D-48149 Münster, Germany

Sustained fear paradigms in rodents have been developed to model clinical situations in patients suffering from long-lasting anxiety disorders. The bed nucleus of the stria terminalis (BNST), as part of the extended amygdala, has been shown to be critically involved in processing of sustained fear responses to more diffuse and unpredictable than discrete and predictable threats. We used a recently established fear conditioning paradigm, which allows the distinction between phasic and sustained states of conditioned fear in non-restrained mice to investigate neuronal and behavioral correlates of phasic/sustained fear.

Thus, we examined different states of conditioned fear (fear-potentiated startle, freezing), induced by predictable or unpredictable training (CS-US timing), on single unit activities in the BNST and on anxiety-like behavior in the elevated plus-maze during fear memory retrieval.

Electrophysiological data, based on intra-BNST unit recordings and a non-biased cluster-analysis, revealed 3 distinct neuronal subpopulations: biphasic-, sustained fear on- and sustained fear off-neurons in the anterolateral BNST. Biphasic- and sustained fear on-neurons indicated the shift from phasic to sustained components of fear, with sustained fear on-cells being activated during sustainment of fear. Behavioral data, based on the elevated plus-maze test, revealed that phasic and sustained states of fear can differentially affect anxiety-like behavior: induction of sustained fear increased anxiety and, induction of phasic fear lead to reduction of anxiety during fear memory retrieval. Our data provide evidence of i) the existence of sustained fear on- and biphasic-neurons confirming the pivotal role of the BNST in processing of sustained fear on the neuronal level, and ii) altered anxiety states using the phasic/sustained fear paradigm in freely behaving mice after predictable or unpredictable CS-US timing.

With this study, we advise the phasic/sustained fear model in rodents to investigate molecular and neuronal mechanisms of phasic and sustained fear with particular focus on the BNST as a target for the development of novel therapeutic strategies. Finally, we want to pro-

mote this animal model as an appropriate translational model for human anxiety disorders.

P12.3
ALTERATIONS OF ULTRASONIC VOCALIZATION (USV) IN PURKINJE CELL SPECIFIC TSC1 KNOCKOUT MOUSE

Wiaderkiewicz J[1,3], Sługocka A[1,2], Głowacka M[1,2], Przybyła M[1,2], Nowacka-Chmielewska M[1,4], Chojnacka D[1] and **Barski JJ**[1,2]

[1]*Department of Experimental Medicine, Medical University of Silesia, ul. Medyków 4, 40-752 Katowice, Poland*

[2]*Department of Physiology, School of Medicine in Katowice, Medical University of Silesia, Katowice, Poland*

[3]*Department of Pharmacology & Physiology, The George Washington University, 2300 Eye St., NW, Washington, DC 20037 USA*

[4]*Laboratory of Molecular Biology, Faculty of Physiotherapy, The Jerzy Kukuczka Academy of Physical Education, Mikolowska 72 a, 40-065 Katowice, Poland*

Mice, and other rodents, are able to communicate using sounds in the ultrasonic range called ultrasonic vocalizations (USV). Quantitative and qualitative analysis of this activity gives information about the social interactions, for example in animal models of autistic spectrum disorders (ASD). USV was analyzed in a mouse model of tuberous sclerosis complex (TSC), where progressive degeneration of Purkinje cells (PC) occurs. TSC is an inherited human disorder with autistic like behavioral manifestations.

To evaluate the influence of PC insufficiency on USV activity, recordings of USV were made in transgenic mouse line lacking expression of hamartin (TSC1) in PC. USV was recorded in newborns from 2–14 postnatal day (PND) with use of the isolation test protocol. Transgenic mouse line used for the experiments was established by PC-specific knockout of the *TSC1* gene. All animal used in that experiment were bred in the animal facility of the Department of Experimental Medicine at the Medical University of Silesia in Katowice. All experimental procedures were planned and performed according to the permission obtained from the Local Committee for Animal Experiments and Welfare.

This approach resulted in a progressive generation of PC in the cerebellar cortex. Analysis of obtained data revealed developmental changes in the USV activity of the pups with some traits characteristic only for the TSC1 knockout animals.

We concluded, that the progressing impairment of PC physiology resulted in disturbance of motor functions of the vocal apparatus and cerebellum dependent alterations of the social behavior.

P12.4
THE FAVORABLE IMPACT OF TIANEPTINE ON THE EVOKED BY PRENATAL STRESS DYSREGULATION OF CHEMOKINE-CHEMOKINE RECEPTOR AXIS IN BRAIN OF ADULT OFFSPRING RATS

Basta-Kaim A, Budziszewska B, Trojan E, Slusarczyk J, Chamera K, Głombik K and Kotarska K

Department of Experimental Neuroendocrinology, Institute of Pharmacology PAS, Cracow 12 Smetna Str, PL 31-343 Cracow, Poland

The impact of stress during pregnancy has received increasing attention. In the CNS the role of chemokines seems to be very intriguing. They are involved not only in neuro-immuno-modulation but also regulation of neurodevelopmental processes. Disturbances in chemokine and their receptors axis may be involved in the pathogenesis of depression.

The aim of present study was to examine whether prenatal stress influence on the CX3CL1 and CXCL12 level in the frontal cortex (Cx) and hippocampus (Hp) of adult rats offspring. The impact of chronic tianeptine administration on this system were evaluated.

Pregnant rats were subjected to restraint stress. At 3 months of age, control and prenatally stressed rats were tested for behavioral changes in Porsolt test. After that male offspring were administered i.p. for 21 days with tianeptine. The animals' behaviour was tested again. The mRNA expression of all analysed chemokines and their receptors was measured by qRT-PCR assay. The protein level was determined by ELISA assay.

Prenatal stress causes long-lasting behavioral alterations expressed as an increase in immobility and a decrease in swimming and climbing time measured in the Porsolt test. Chronic treatment of tianeptine normalized all above-mentioned changes in prenatally stressed offspring. Prenatal stress diminished mRNA and protein of CX3CL1 and CX3CR1 in the Cx and Hp. In brain of prenatally stressed rats expression of CXCR4 was decreased and levels of CXCL12 and its receptor CXCR7 were elevated. The chronic tianeptine administration attenuated all evoked by stress changes in chemokine-chemokine receptor axis.

Prenatal stress procedure leads not only to persistent behavioral disturbances but also malfunction in brain chemokine-chemokine receptors network. Brain chemokines systems can be indicate as a potential attractive target for antidepressant drug action.

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P12.5
EFFECT OF CO-TREATMENT WITH ARIPIPRAZOLE AND ANTIDEPRESSANTS ON THE MK-801-INDICED CHANGES IN THE OBJECT RECOGNITION TEST IN RATS

Rogóż Z[1,2], G Skuza[1], Wąsik A[1] and Lorenc-Koci E[1]
[1]*Institute of Pharmacology, Polish Academy of Sciences, Krakow, Poland*
[2]*The Podhale State Higher Vocational School, Nowy Targ, Poland*

Schizophrenia is a devastating psychiatric disorder that impairs mental and social functioning and affects approximately 1% of the world's population. It is known that in contrast to pharmacotherapy with typical antipsychotics, atypical antipsychotic agents alleviate not only the positive symptoms of schizophrenia but also the negative ones, but those effects are small and mechanisms of this action are still unknown. A few clinical reports have suggested that antidepressant drugs are able to augment the activity of atypical antipsychotic drugs, thus effectively improving treatment of the negative and some cognitive symptoms of schizophrenia.

In the present study, we aimed to evaluate the effect of antidepressants (escitalopram or mirtazapine) and aripiprazole (an atypical antipsychotic drug) given separately or jointly, on the behavioral deficits induced by MK-801 (a NMDA receptor antagonist) given prior to the first introductory session, in the object recognition memory test. The experiments were conduced in a black polyvinyl chloride box ($57 \times 67 \times 30$ cm). Male Sprague-Dawley rats (230–250 g) were tested for the ability to discriminate between an old, familiar and a novel object. Antidepressants and aripiprazole were given 30 min before MK-801, and MK-801 was administered 30 min before the first introductory session. Memory retention was evaluated for 5 min, starting 60 min after the introductory session.

The present results showed that MK-801 (0.1 mg/kg) decreased memory retention when given before the introductory session. Aripiprazole (0.3 and 1.0 mg/kg) reversed that effect. Co-treatment with an inactive dose of aripiprazole (0.1 mg/kg) and escitalopram or mirtazapine (5.0 mg/kg, but not 2.5 mg/kg) abolished the deficit of object recognition memory induced by MK-801.

The obtained results suggest that antidepressants may enhance the antipsychotic-like effect of aripiprazole in the animal test used for evaluation of some cognitive symptoms of schizophrenia.

This study was financially supported by statutory funds of the Institute of Pharmacology, Polish Academy of Sciences, Kraków, Poland.

P12.6
THE ANTIDEPRESSANT EFFECTS OF KETAMINE ON THE LATERAL HABENULA AND THE BEHAVIOUR OF NORMAL, RESTRAINT STRESS AND MATERNALLY DEPRIVED RATS

Crews-Rees A[1,2], Pierucci M[1], Delicata F[1], Benigno A[3] and Di Giovanni G[1,2]
[1]*Department of Physiology and Biochemistry, Faculty of Medicine and Surgery, University of Malta - Msida, Malta*
[2]*School of Biosciences, Cardiff University - Cardiff, U.K*
[3]*Department of Experimental Biomedicine and Clinical Neurosciences, Human Physiology Section "Giuseppe Pagano", University of Palermo - Palermo, Italy*

Depression accounts for around 800,000 deaths globally every year, therefore there is a need to produce an antidepressant with a rapid onset of action and sustained effects, which is shown with ketamine. Ketamine, a NMDA antagonist, has been linked with the decrease of neuronal activity in the lateral habenula (LHb), which is an important structure in modulating the midbrain's monoaminergic system, and is typically hyperactive in depressed patients. The aim of this study is to investigate the antidepressant effects of ketamine and citalopram in normal rats and in animal models of depression by using a behavioural and electrophysiological approach.

Control rats, randomly assorted into 3 groups received either ketamine 10 mg/kg, citalopram 10 mg/kg or saline 1 ml/kg ($n = 10$ for each group) and underwent to forced swim test (FST) and elevated plus maze (EPM) test. Following the behavioural study, cells-per-track experiments were performed in the same rats to investigate the effects of these treatments on the LHb basal neuronal activity, by using standard *in vivo* single unit electrophysiology. Moreover, the effect of intravenous ketamine (0.25–8.0 mg/kg; $n = 20$) on the LHb neuronal activity was investigated in control and chronically-stressed rats (an animal model of depression) by using extracellular *in vivo* recording technique.

Preliminary results in normal rats show that ketamine and citalopram induced an antidepressant-like effect in the FST, as well as a decrease in anxiogenic behaviour in the EPM test. In chronically-stressed rats, ketamine produced a reduction of LHb basal firing rate by greater than 50%.

These results show that ketamine potentially has an antidepressant effect, as well as causing inhibition of

the LHb neuronal activity of the depressed rats. Future studies need to be conducted in order to understand if these two phenomena are mere epiphenomena or causally correlated to be involved in the antidepressant mechanism of ketamine.

P12.7
A NEUROIMAGING STUDY ABOUT EMOTIONAL PERSPECTIVE-TAKING: AN FMRI STUDY

Son JW
Department of Neuropsychiatry, College of Medicine, Chungbuk National University, Cheongju, Republic of Korea

Perspective-taking is one of social cognitive function, and the ability of taking either one's own perspective or the perspective of others is very important for people to live in social community. This study aimed to investigate the difference of brain activity in viewing common emotional situation according to perspective-taking.

Using fMRI, brain activities were measured while performing the task viewing common emotional situation (happy, anger, sad, neutral) on either his own perspective (self-perspective) or the perspective of his mother (third-person perspective) in fourteen healthy men. The relatively activated brain areas on either self-perspective or third-person perspective were investigated, then the relationship between the activated brain region and the scores of self report about some emotion or empathic ability were explored.

The relatively activated brain area on self-perspective were bilateral paracentral lobule (BA 5), right postcentral gyrus (BA 3), right precentral gyrus (BA 4), left superior temporal gyrus (BA 22), left medial frontal gyrus (BA 6), whereas on third-person perspective were right inferior frontal gyrus (BA 47), left caudate body and tail, right superior temporal gyrus (BA 38), right medial frontal gyrus (BA 8). The relative activity of left superior temporal gyrus on self-perspective was positively correlated with the score of Beck Depression Inventory.

This study demonstrated that the activated brain region according to perspective-taking were different while viewing common emotional situation. The depressive feeling would have influence on the brain activity related to perspective-taking.

P12.8
BRAIN MORPHOLOGY AND FUNCTIONAL CHANGES ASSOCIATED WITH VISUAL SEXUAL AROUSAL IN MENOPAUSAL WOMEN

Kim G-W and Jeong G-W
Department of Radiology, Chonnam National University Medical School, Gwangju, Republic of Korea

This study utilized a combined use of fMRI and voxel-based morphometry (VBM) to assess the brain morphological and functional alterations in menopausal women. Twenty premenopausal women and 20 menopausal women underwent functional and structural MRI. Brain functional activity was measured while viewing an erotic video. The activation maps were obtained from the contrast of 30 sec sexual activation period versus 30 sec neutral period. An analysis of covariance (ANCOVA) adjusting for age was used to evaluate the differential functional and morphological alterations between the two groups. Compared with premenopausal women, menopausal women showed significantly decreased activity in the precentral gyrus, orbitofrontal gyrus (OFG), superior frontal gyrus, and dorsolateral prefrontal cortex while viewing the erotic video clips ($p < 0.005$). In morphometric analysis, menopausal women exhibited significantly decreased gray matter (GM) volumes of the supplementary motor area, OFG, middle temporal gyrus, and insula and decreased white matter (WM) volume of the precentral gyrus compared with premenopausal women ($p < 0.005$). In addition, the GM volume changes in the OFG were positively correlated with blood-oxygen-level-dependent (BOLD) signal changes in the OFG during sexual arousal in menopausal women. This study highlights the relationship between menopause-related morphological and functional alterations in menopausal women. The decreased GM/WM volumes and reduced functional activity together are closely associated with the symptoms of menopause. This research was supported by the NRF grants funded by Korean Government, the Ministry of Education (2014R1A1A2006730) and MSIP (2015R1A2A2A01007827).

P12.9
THE LONG TERM BRAIN EFFECTS OF BINGING ON ALCOHOL AND MARIJUANA IN ADOLESCENT TOBACCO USERS: A STUDY ON MOTIVATION IN OPERANT FOOD-REINFORCED RESPONDING; 11THE PACEVILLE PROJECT: II"

Abela N[1], Pierucci M[1], Haywood K[1,2], Casarrubea M[3], Vella M[1], Crescimanno G[3], Benigno A[3] and Di Giovanni G[1,2]
[1]*Laboratory of Neurophysiology, Department of Physiology and Biochemistry, Faculty of Medicine and Surgery, University of Malta - Msida, Malta*
[2]*School of Biosciences, Cardiff University - Cardiff, U.K*
[3]*Department of Experimental Biomedicine and Clinical Neurosciences, Human Physiology Section "Giuseppe Pagano", University of Palermo - Palermo, Italy*

This study is focused on the long term effects on motivation of nicotine administration together with binging on cannabinoid and alcohol during adolescence in Long Evans rats. This research focuses on the effect of this combination of drugs, which is on the increase in human adolescents, on the developing brain. P30 males (treatment and vehicle groups, $n = 10$ each) and females (treatment and vehicle groups, $n = 10$ each) rats were treated daily with 1 mg/kg of nicotine intraperitoneally for 28 days, together with twice weekly (Binging Days) for four weeks intraperitoneal injections of 1.2 mg/kg WIN55,212-2 mesylate and 3 g/kg ethanol via gavaging or with their vehicles respectively. At P90 these rats underwent a starvation regime and after reaching an approximately 85% of ad libitum body weight were tested for six consecutive days on a FR1 schedule followed by a 42 days of rest and then another six days of daily FR2 schedule testing. Latency and response for the task completion were recorded for each rat.

Results revealed that in the FR1 schedule, treated female rats' rate of response was significantly less than female control rats, during day 3 ($\chi^2(1) = 4.341$, $p = 0.037$), 4 ($\chi^2(1) = 6.874$, $p = 0.009$), 5 ($\chi^2(1) = 4.056$, $p = 0.044$) and 6 ($\chi^2(1) = 4.229$, $p = 0.040$). A lower rate of response by treated females was also produced during the 6th day FR2 schedule ($\chi^2(1) = 4.977$, $p = 0.026$) when compared to control females. Control and Treated males did not show any significant difference in FR1 and FR2 schedules. Significant difference was observed in day 3 ($\chi^2(1) = 2.301$, $p = 0.050$), 4 ($\chi^2(1) = 4.056$, $p = 0.044$), 5 ($\chi^2(1) = 4.341$, $p = 0.037$) and 6 ($\chi^2(1) = 3.598$, $p = 0.050$) of the FR1 schedule between treated males and treated females, with evident impairment in females.

The combined treatment with cannabinoids, alcohol and nicotine in adolescence affected more adult female rats compared to males, revealing them at first glance a gender-related alteration of motivation system.

P12.10
LORCASERIN, A SEROTONIN2C/2A RECEPTOR AGONIST, PREFERENTIAL MODULATES MESOLIMBIC VS. NIGROSTRIATAL DOPAMINERGIC FUNCTION: AN *IN VIVO* ELECTROPHYSIOLOGICAL AND MICRODIALYSIS STUDY

Ramos M[1], Pierucci M[1], Delicata F[1], Manem J[2], Benigno A[3], De Deurwaerdere P[2] and Di Giovanni G[1,4]
[1]*Laboratory of Neurophysiology, Department of Physiology and Biochemistry, Faculty of Medicine and Surgery, University of Malta - Msida, Malta*
[2]*CNRS, Institut des Maladies Neurodégénératives, UMR 5293, F-33000 Bordeaux, France*
[3]*Department of Experimental Biomedicine and Clinical Neurosciences, Human Physiology Section "Giuseppe Pagano", University of Palermo - Palermo, Italy*
[4]*School of Biosciences, Cardiff University - Cardiff, U.K*

Serotonin-2C Receptors (5-HT_{2C}Rs) have been associated with numerous physiological responses including mood disorders, drug abuse and feeding behaviour. Very limited pharmacotherapeutic options exist to selectively target the 5-HT_{2C}Rs. Lorcaserin is a highly selective 5-HT_{2C}R agonist that has been recently approved by the FDA for the treatment of obesity disorders. The effect of lorcaserin on dopaminergic function has not yet been investigated, even though serotonin (5-HT) is known to be a major modulator of dopamine (DA) neural activity and 5-HT_{2C}Rs might be a principal mechanism by which 5-HT inhibits DA function. The aim of this study is to analyse the effect of lorcaserin on the neuronal activity of DA cells of the ventral tegmental area (VTA) and substantia nigra pars compacta (SNc), as well as its effect on DA terminal release at the Nucleus Accumbens and Striatum.

The neuronal activity of single DA cells was recorded by standard extracellular recordings from the VTA and SNc in anaesthetised SD rats. Lorcaserin was administered in cumulative doses (5–640 µg/kg, i.v.) fashion. In the antagonism experiments, the 5-HT_{2C}R selective antagonist SB242084 (200 µg/kg, i.v.) was given 5 min before lorcaserin. The effect of lorcaserin (3–10 mg/kg) on extracellular monoamine and metabolite levels were measured using online microdialysis and the tissue levels of DA and metabolites were assessed using HPLC.

Results showed that lorcaserin ($n = 10$) significantly reduced ($p < 0.05$) the firing rate of DA neurons of the VTA when compared to controls (saline, $n = 10$). However, lorcaserin had no significant effect on the firing rate of SNc DA neurons ($n = 10$). Preliminary results in microdialysis experiments confirmed the lack of effect of lorcaserin on striatal dopamine release.

5-HT_{2C}Rs are involved in the 5-HT mediated inhibition of the DA function. Moreover, this data shows that the 5-HT_{2C}R-mediated inhibition is specific to the mesocortical limbic dopaminergic system rather than to the nigrostriatal system. In addition, this study suggests lorcaserin as a potential drug in the treatment of compulsive behaviours and drug abuse by preferentially inhibiting the VTA DA system.

P12.11
TEMPORAL STRUCTURE OF THE MUSCULAR DYSTROPHY X-LINKED MOUSE BEHAVIOR TESTED IN OPEN FIELD

Faulisi F, Raso G, Morici G, Benigno A, Crescimanno G and Casarrubea M

Department of Experimental Biomedicine and Clinical Neurosciences, Human Physiology Section "Giuseppe Pagano", University of Palermo - Palermo, Italy

Duchenne muscular dystrophy (DMD) is a severe X-linked recessive disease where the cytoskeletal protein dystrophin is not expressed in muscle as well as in the nervous system. The dystrophin deficient mdx (muscular dystrophy X-linked) mouse is genetically comparable to the human form of DMD and it is the most used animal model. Despite the importance of the central deficits of the DMD (e.g. deficits in cerebellar circuitry has been observed) only little information has been obtained, until now, on the behavioral structure of this mouse strain. To shed light on this matter, 2 different groups of mice, 5 wild type (WT) and 5 mdx were tested for 10 min in open field (OF) and their behavior recorded using a digital camera. Both quantitative and T-pattern analyses (TPA) were applied. TPA is a multivariate technique based on the assessment of critical relationships among the events in the course of time.

Quantitative analysis shows a total of 1073 events for WT mice whereas 1657 for mdx mice with an increase in walking activities and vertical explorations for the last group; in addition a reduction in static activities was observed as well. Concerning TPA, WT mice show 26 different T-patterns while mdx mice show 153 different T-patterns. As to WT mice, mean occurrences ± SE of T-patterns are 101.27 ± 16.55 with a mean length ± SE of 2.54 ± 0.13; as to mdx mice, mean occurrences are 30.33 ± 4.87 and mean length 10.42 ± 0.52.

These preliminary results suggest that the behavior of the two strain of mice tested in the OF apparatus has a complex structure characterized by close interrelationships occurring sequentially and with significant constraints on the interval lengths separating them. In comparison with WT, mdx mice show a different behavioral organization with a more articulated structure of the temporal patterns. Present study sheds light, for the first time, on specific temporal features of behavior in an animal model of DMD.

P12.12
EPILEPSY AND AUTISM COMORBIDITY: ROLE OF *GAIN-OF-FUNCTION* DEFECTS OF Kir4.1 CHANNELS

Sicca F[1], Ambrosini E[2], Cozzolino O[3], D'Adamo MC[4], Marchese M[1], Minutolo F[5], Pessia M[6], Tuccinardi T[5], Valvo G[1], Ratto GM[3] and Santorelli FM[1]

[1]*Department of Developmental Neuroscience, IRCCS Fondazione Stella Maris, Pisa, Italy*

[2]*Department of Cell Biology and Neuroscience, Istituto Superiore di Sanità, Rome, Italy*

[3]*NEST, Istituto Nanoscienze CNR and Scuola Normale Superiore, Pisa, Italy*

[4]*Department of Physiology & Biochemistry, University of Malta, Msida, Malta*

[5]*Department of Pharmacy, University of Pisa, Pisa, Italy*

[6]*Department of Experimental Medicine, University of Perugia, Perugia, Italy*

Dysfunctions of the astrocytic inwardly-rectifying potassium channel Kir4.1 (*KCNJ10*) result in impaired control of extracellular K+ in the brain contributing to pathogenic mechanisms underlying Autism-Epilepsy Phenotype (AEP), a condition where seizures (or EEG abnormalities) and Autism Spectrum Disorders (ASD) coexist.

To define the role of Kir4.1 variants in AEP, we sequenced *KCNJ10* in a sample of affected individuals, and performed genotype-phenotype correlations. The effects of mutations on channel activity, protein trafficking, and astrocyte function, were investigated in *Xenopus laevis oocytes*, and in human astrocytoma cell lines. An *in vivo* model of the disorder has also been explored by assessing locomotor behaviour, EEG recordings, and two-photon brain imaging of *KCNJ10a* morphant zebrafish overexpressing the mutated human *KCNJ10*.

Germline heterozygous *KCNJ10* variants were found in about 5% of affected children, mainly displaying epileptic spasms and sensory processing dysregulation. All pathogenic variants revealed *gain-of-function* defects when investigated on *in vitro* cell systems. Kir4.1 mutations also recapitulated the main disease phenotype when transiently modelled *in vivo* in zebrafish embryos.

Our findings confirm that variants in *KCNJ10* deserve attention in autism-epilepsy, and provide insight into the molecular mechanisms of autism and seizures, as well as into the role of astrocyte dysfunction in abnormal synaptic transmission and electrical discharge underlying the disorder. Further work on zebrafish models is now ongoing to get larger phenotype assessments and high-throughput drug screenings, to allow focusing studies in transgenic mammalian models while seeking for new drugs for children with autism-epilepsy comorbidity.

P12.13
THE FAAH INHIBITOR NF1245 SHOWS ANTIEPILEPTIC EFFECTS IN TWO RAT MODELS OF EPILEPSY

Stockton E[1,2], Colangeli R[1], Di Maio R[3], Pierucci M[1], Benigno A[4], Brindisi M[5], Grillo A[5], Gemma S[5], Campiani G[5], Maccarrone M[6], Minetti P[7], Vella M[1], Butini S[5] and Di Giovanni G[1,2]

[1]*Department of Physiology and Biochemistry, Faculty of Medicine and Surgery, University of Malta - Msida,*

Malta
[2]*School of Biosciences, Cardiff University - Cardiff, U.K*
[3] *Dept. Neurology University of Pittsburgh Pittsburgh, USA*
[4]*Department of Experimental Biomedicine and Clinical Neurosciences, Human Physiology Section "Giuseppe Pagano", University of Palermo - Palermo, Italy*
[5]*Department of Biotechnology, Chemistry and Pharmacy European Research Centre for Drug Discovery and Development University of Siena, Italy*
[6]*Department of Medicine - Campus Bio-Medico University of Rome, Italy*
[7]*Sigma-Tau Industrie Farmaceutiche Riunite S.p.A. Via Pontina Km 30, 400, 00040 Pomezia, Italy*

Temporal lobe epilepsy (TLE) is the most common form of epilepsy accounting for around 60% of all cases and although antiepileptic drugs are available they are ineffective in up to a third of patients. Thus there is a need for the development of new treatments. The endocannabinoid system has long been implicated in the generation of epilepsy due to reduced expression of CB1 receptors in several models of epilepsy, as well as a decrease in the levels of the endogenous cannabinoid anandamide (AEA) in human patients with epilepsy. Anandamide has also been associated with an on demand protective response to seizures. As a result, modulation of the endocannabinoid system has become a focus of research into epilepsy and CB1 agonists such as WIN 55,212-2 have been shown to have antiepileptic effects, although do not address the issue of detrimental effects to memory and cognition in general. A different approach to modulating the endocannabinoid system is to elevate the levels of the two endogenous cannabinoids, AEA and 2-arachadonylglycerol (2-AG) by blocking their enzymatic breakdown with selective inhibitors.

The aim of our project is to evaluate the antiepileptic effects of a newly developed fatty acid amide hydrolase (FAAH) inhibitor, NF1245 (also named ST3913), in both pilocarpine-induced status epilepticus (SE) and Maximal Dentate Activation (MDA) model of mesial TLE in Sprague-Dawley rats.

NF1245 (10 mg/kg; i.p. $n = 10$) administered 45 minutes prior to pilocarpine (360 mg/kg, i.p.) significantly reduced the severity of SE seizures scored on the Racine scale and also increased the time to onset of SE whilst avoiding post SE thiol oxidation damage in the hippocampus at 24 h. In the hippocampal MDA model the same dose of NF1245 10 mg/kg i.p. decreased the elongation of MDA compared to control animals ($n = 6$, for both groups) when administered after 1 hour of the elicitation of the MDA.

Preliminary findings show that inhibiting the FAAH enzyme may be a new therapeutic approach to treat patients with TLE.

P12.14
RECURRENT PATTERN DISCOVERY IN D1CT-7 MOUSE MODEL OF TIC-RELATED BEHAVIOR

Santangelo A[1], Bortolato M[2], Di Giovanni G[3,4], Ricca V[1], Crescimanno G[5], Benigno A[5] and Casarrubea M[5]
[1]*Department of Neuroscience, Psychology, Drug Research and Child Health, Psychiatry Unit, University of Florence, Florence, Italy*
[2]*Department of Pharmacology and Toxicology, Interdepartmental Neuroscience Program, University of Utah, 30 S 2000 E, Skaggs Hall, Room 3916, Salt Lake City, UT, 84112, USA*
[3]*Department of Physiology and Biochemistry, Faculty of Medicine and Surgery, University of Malta, Malta*
[4]*Neuroscience Division, School of Biosciences, Cardiff University, Cardiff, U.K*
[5]*Department of Biomedicine and Clinical Neurosciences, Laboratory of Behavioral Physiology, Human Physiology Section "Giuseppe Pagano", University of Palermo, Palermo, Italy*

Transgenic D1CT-7 mice represent a pathophysiologically-grounded translational model of tic-related disorders and compulsivity, characterized by tic-like hyperkinetic vertical behaviors such as head-body twitches (HBT) and deficits in sensorimotor gating. These mice show a selective hyperactivity of cortico-striato-thalamo-cortical circuitry.

Aim of this study was to investigate the influence of such a neurobiological construct on the display of behavioral motor sequences of D1CT-7 mice tested in the open field (OF).

We tested 5 adult experimentally-naïve D1CT-7 mice and 5 wild-type controls (WT) in OF for 10 min. Video files were processed through behavioral coding. An ethogram of 13 behavioral elements, including commonly-reported mouse behaviors plus HBT, was employed. Temporal Pattern Analysis (TPA) was performed to detect behavioral sequences. TPA is a refined tool to determine whether two or more behavioral elements occur sequentially and with statistically significant time intervals. A mean number of 830.4 elements were identified in D1CT-7 mice and 464 in WT (t-test: $t_{(8)} =, p < 0.001$). This result was associated with a greater vertical activity and walking.

However, TPA did not show a consistent difference in number of behavioral patterns. We found 23 different patterns in D1CT-7 group and 26 in WT, occurring respectively 4091 and 4568 times.

From a qualitative perspective, WT showed only 5 different patterns of vertical activity recurring 440 times, whereas D1CT-7 displayed comprehensively 13 pat-

terns of vertical activity recurring 1934 times. Among these, 1122 patterns encompassed perseverative climbing, HBT or rearing. Walking-HBT pattern occurred 348 times in D1CT-7 and was not found in WT. Besides, D1CT-7 did not display any pattern including stretched-sniffing component, a key novelty-related behavior that is, in turn, widely found in WT patterns.

D1CT-7 model summarizes some pivotal aspects of tic-related disorders. Accordingly, TPA showed a marked organization of behavioral sequences around vertical activities, suggesting perseverative features. The absence of stretched-sniffing containing patterns may be associated with impaired inhibition upon intrusive vertical activities. Further analyses are underway in our laboratories.

P12.15
IMPACT OF BINGE-LIKE ETHANOL INTOXICATION DURING ADOLESCENCE ON VULNERABILITY TO ANXIETY AND DEPRESSIVE DISORDERS AT ADULTHOOD IN WISTAR RATS

Hicham El M, Tarik T, Abderrahim L, Ali O, Aboubaker E and Abelhalim M
Laboratory of Genetics, NeuroEndocrinology and Biotechnology, Department of Biology, Faculty of Sciences, Ibn Tofail University, Kenitra Morocco

Adolescent alcohol binge drinking constitutes a major vulnerability factor to develop psychiatrics diseases such as depression and anxiety. However, mechanisms underlying this susceptibility remain unknown. We evaluated, in adulthood, the effect of adolescent binge-like ethanol intoxication on vulnerability to anxiety-depressive behavior in Wistar rats. To model binge-like ethanol intoxication, every 2 days, rats received an ethanol injection (3.0 g/kg) for 2 consecutive days across 14 days either from postnatal day 30 (PND30) to 43 (early adolescence). In late adolescence animals (from PND45), we measured free ethanol consumption in the two-bottle choice paradigm, motivation for ethanol, both ethanol's rewarding and aversive properties in the conditioned place preference (CPP) and taste aversion (CTA) paradigms.

In young adult animals, the intoxicated rats were exposed, for 6 consecutive weeks (from PND 46 to 88th), to an unpredictable chronic light stress (based on subjecting rats to a period of mild socio-environmental stressors) modeling depression-like behavior. From the 90th PND, the depressive rats phynoque'll be evaluate by, measurement of the activity in the familiar environment, quantification of the peeling condition, evaluation of a resignation behavior (one using the forced swim test FST and the tail suspension test TST), measuring a major criterion in the diagnosis of depression "anhedonia" bay using the sucrose preference test (SPT) or novelty suppressed feeding test (NSF). The memory and learning disorders, and the oxidative status of the brain (lipid peroxidation, catalase activity and superoxide dismutase) were also measured in adulthood.

Preliminary results of this work have shown the establishment of depressive-like affective disorders and cognitive disorders (spatial memory) in animals previously treated with ethanol. Some other results of this study, in the course of exploitation, will be communicated during symposium.

P12.16
NEUROCHEMICAL AND BEHAVIOURAL EFFECTS OF COCAINE IN HATANO HIGH AND LOW-AVOIDANCE RATS

Piras G[1,2], Pisanu A[3], Lecca D[1,2], Serra GP[1], Valentini V[1,2] and Di Chiara G[1,2,3]
[1] *University of Cagliari, Dept. of Biomedical Sciences, Cagliari, Italy*
[2] *National Institute of Neuroscience (INN), Cagliari, Italy*
[3] *Institute of Neuroscience, National Research Council of Italy*

Hatano high- (HAA) and low-avoidance (LAA) strains were selected from Sprague–Dawley rats on the basis of their different performance in the shuttle-box task at the Hatano Research Institute (Japan) in 1985. Several studies have highlighted differences among HAA and LAA rats in the performance of other tasks (i.e. spontaneous motor activity) and in physiological parameters during and/or after stress conditions exposure. The present study was aimed to investigate among Hatano strains: 1) dopamine (DA) transmission responsiveness in the shell and core of the nucleus accumbens (NAC) induced by cocaine; 2) cocaine self-administration (SA) behavior.

To these aims, we used in both strains: 1) *in vivo* brain microdialysis to study the effect of cocaine (5–10 mg/kg, ip) on DA transmission in the shell and core of NAC and 2) we assessed the ability of cocaine (0.1-0.2-0.4 mg/kg/infusion) to maintain SA in fixed (FR1 and FR5) and progressive ratios (PR3-4) schedules of reinforcement.

No strain or brain area-related differences in basal DA output were obtained. Acute ip cocaine administration induced a larger DA increase in the shell of LAA compared to HAA rats, whereas the opposite was observed in the core compartment. LAA but not HAA maintained cocaine SA at the dose 0.4 under FR1 schedule. Under FR5 and $PR_{3\text{-}4}$ schedules both strains self-administered cocaine 0.4, however active nose poking, infusions and drug intake were greater in LAA than HAA. The break-

ing point reached by LAA strain was significantly higher compared to HAA strain. No differences were observed during extinction among strains.

Our results showed that Hatano strains differ in their neurochemical and behavioral responses to cocaine. Moreover, these data supported the hypothesis that NAC shell DA is important for the rewarding and reinforcing properties of cocaine. Further studies in Hatano rats could clarify the role of the DA projections to NAC shell and core and genetic factors in the mechanism of drug abuse.

P12.17
PERINATAL EXPOSURE TO LEAD (PB) INDUCES ALTERATION IN GLYCOGEN METABOLISM IN DEVELOPING BRAIN OF RAT OFFSPRING

Baranowska-Bosiacka I[1], Gutowska I[2], Falkowska A[1], Łukomska A[2], Kolasa A[3], Pilutin A[3], Metryka E[1], Kupnicka P[1], Goschorska M[1], Chlubek D[1]
[1]*Department of Biochemistry*
[2]*Department of Biochemistry and Human Nutrition*
[3]*Department of Histology and Embryology, Pomeranian Medical University in Szczecin, Poland*

Previous studies have provided evidence for an association between the elevated levels of lead (Pb) in the blood in children and impaired memory, concentration, learning, and lowered IQ. However, the molecular mechanism of these changes is not fully understood. Importantly, even the acceptable blood Pb concentrations in children have been shown to alter many biochemical processes in the neuronal tissue, affecting storage processes, release of neurotransmitters, signaling pathways, and energy metabolism of neurons. All these changes may result in serious neurological disorders. Glycogen plays a key role in the brain energy metabolism; it is responsible for the correct cooperation between the neuron and astrocyte, essential for the proper brain plasticity and neurotransmission. In this study, we aimed at evaluating the effect of exposure of rats to Pb in the pre- and neonatal periods on the expression of glycogen metabolism enzymes: glycogen synthase, glycogen synthase kinase, glycogen phosphorylase, and connexin 43 – an enzyme directly responsible for the metabolic cooperation between neurons and astrocytes. The study was conducted on young rats which were given 0.1% lead acetate in utero and when fed by mother, which resulted in the whole blood Pb levels below 10 mg/dL, i.e. below the previously acceptable limits for humans. This study showed an increase in the concentration of glycogen in the prefrontal cortex, hippocampus, and cerebellum in the experimental animals compared to controls. Pb intoxication also resulted in the reduction in the expression of synthase and glycogen phosphorylase proteins in all tested brain regions. We also observed the downregulation of connexin 43 protein in all brain regions of rats exposed to Pb. There was a statistically significant increase in the expression of the phospho-GSK-3β (Tyr216) by approx. 25% in the prefrontal cortex and by approx. 30% in the cerebellum compared to controls. We observed no significant change in the expression for GSK3β phosphorylated at Ser 9 in any of the tested brain regions.

1) Pre- and neonatal exposure to Pb, resulting in blood levels previously considered acceptable for humans, affects glycogen levels and expression of enzymes directly involved in glycogen synthesis and degradation in the developing rat brain.
2) Through the disorder of connexin protein expression Pb may impair the flow of lactate (a glycogen metabolite) between a neuron and astrocyte, thus disrupting energy metabolism of neurons.

P12.18
THE DIFFERENCE IN REACTIVENESS OF ALPHA1 ADRENERGIC RECEPTOR SUBTYPES AFTER TWENTY FOUR HOURS EXPOSURE TO ANTIDEPRESSANT DRUGS

Kusmierczyk J, Chmielarz P, Rafa-Zablocka K, Kowalska M and Nalepa I
Department of Brain Biochemistry, Institute of Pharmacology, Polish Academy of Sciences, Krakow, Poland

The adrenergic α1 receptor (α1-AR) system is involved in mechanism of antidepressant action, and its role in depressive disorders is also postulated. The α1-AR includes three subtypes, but the specific functional role of particular receptor subtype in the brain remains unclear. It was shown that imipramine exhibits a differential potency toward α1A- or α1B-AR subtypes. Furthermore studies on transgenic animals suggest different functional role of this receptor subtypes in depression. Aim of the study was to investigate if and how prolonged incubation with antidepressant drugs (citalopram (CIT), fluoxetine, reboxetine, mianserin (MIA), imipramine (IMI), desipramine (DMI)) could affect the α1A-, α1B-, α1D- AR reactiveness *in vitro*.

PC12 cells with stable expression of α1A- or α1B-AR and Ready-to-Assay™ α1D AR cells were seeded in 60% confluence and were incubated in standard growth medium with addition of 10 µM drug concentration. After 24 h cells were washed and harvested for IP1 (of α1A- or α1B-AR cells) or Ca^{2+} (α1D AR cells) measurement after noradrenaline (NOR) stimulation.

We found the subtype specific differences in response to NOR after 24 h preincubation with drugs. In example

DMI and IMI shifted dose response curve rightwards in case of α1A-AR (EC 50 from 15 nM to 224 and 39 nM respectively), but increased maximal stimulation of α1B- (to 230% of control level) and α1D-AR (127%). CIT increased maximal stimulation of α1A- (155%) and α1B-AR (138%) but did not show any effect in α1D-AR. In contrast to CIT, MIA augmented the maximal response to NOR (130%) and shifted dose response curve rightwards of α1D-AR (from 1.45 to 2.74 nM) with no effect in other receptor subtypes.

The differential susceptibility of α1-AR to antidepressant action is interesting in the light of reports of different role of these receptors in depressive-like behaviors in mice.

P12.19
ADOLESCENCE VERSUS ADULTHOOD: DIFFERENCES IN BASAL DOPAMINE TRANSMISSION AND RESPONSE TO DRUGS OF ABUSE

Corongiu S[1], Dessì C[2] and **Cadoni C**[2]
[1] *University of Cagliari, Department of Biomedical Sciences, Neuropsychopharmacology Section, Cagliari, Italy*
[2] *CNR Neuroscience Institute, Cagliari, Italy*

Adolescence is a crucial period of brain development where important physiological, neurobiological, and cognitive changes take place. This stage of life is characterized by a heightened vulnerability to drug abuse, as well as being the peak time for clinical onset of most mental illnesses. Current theories posit that typical adolescent behavior is due to an imbalance between activity of cortical and sub-cortical areas. Dopamine (DA) system is particularly involved in this stage of development being a key player in reward circuitries and incentive-motivated approach behavior, as well as in decision making processes. While there is large agreement in a delayed maturation of DA mesocortical system, it is still debated if mesolimbic and mesostriatal systems of adolescents are hyper- or hypo- reactive. The aim of our study was to directly evaluate differences in mesolimbic and nigrostriatal DA transmission between adults and adolescents rats and its responsiveness to different drugs of abuse through *in vivo* microdialysis.

Male Sprague-Dawley rats 5–7 or 10–12 old were implanted with dual probe, aimed at the shell and core of NAc or dorsolateral striatum (DLS) and challenged with nicotine, Δ^9-tetrahydrocannabinol (THC), cocaine, or morphine and extracellular DA levels monitored simultaneously with behavior.

Although no significant difference in basal DA levels in the NAc was observed between adolescents and adults, adolescents showed significant lower basal DA levels than adult rats in DLS. While no difference was observed in the effect of cocaine in the shell and core of NAc, a greater DA increase was observed in DLS of adolescent rats. An increased DA response was observed in the NAc shell following nicotine, THC, and morphine in adolescent as compared to adult rats. Moreover, behavioral activation differed between adolescent and adult rats.

The above differences in drugs effects might be explained by developmental changes in the endocannabinoid and opioid systems, as well as by differential expression of nicotinic receptors during brain development.

In conclusion these results while adding further insight in the development of the reward system during different stages of adolescence, provide a likely explanation for the gateway effect of nicotine and THC toward abuse of other illicit substances.

P12.20
CHARACTERIZATION OF ENDOCANNABINOID SYSTEM IN AN ANIMAL MODEL OF BINGE EATING

Pucci M[1], Micioni Di Bonaventura MV[2], Bellia F[1], Falconi A[1], Maccarrone M[3], Cifani C[2] and D'Addario C[1]
[1] *University of Teramo, Biosciences, Teramo, Italy*
[2] *University of Camerino, School of Pharmacy, Camerino, Italy*
[3] *Campus Biomedico, Department of Medicine, Rome, Italy*

Stress, together with dieting and negative affects, is a common trigger of eating disorders. Binge-eating (BE) disorder is characterized by the consumption of an unusual large amount of food associated with the sense of loss of control over eating during the episode. The physiological control of BE is extremely complex, involving a balance of both central and peripheral neurotransmitters and neuropeptides that interact to stimulate or inhibit food intake. The endocannabinoid (eCB) system has long been known as a modulator of physiological functions and plays an important role in brain circuits related to feeding behaviours.

We analyzed the transcriptional regulation of eCB system genes in selected brain regions (Amygdala complex, Caudate Putamen (CP), Nucleus Accumbens, Hypothalamus (HY) and Ventral Tegmental Area) of an animal model of BE which combines cycles of food restriction/refeeding and acute stress to evoke BE for sweet high palatable food.

Analysis in the HY of stressed and exposed to restriction rats revealed a significant selective decrease of Fatty Acid Amide Hydrolase mRNA when compared to the other groups.

Moreover in the CP of stressed rats exposed or not to restriction it was observed a significant decrease of gene

expression of both Cannabinoid receptor type-2 and type-3 respect to no stressed rat as well as of Vanilloid Receptor 1 in the stressed and restricted group respect to non-stressed and non-restricted rats. No changes were observed in the other brain regions analyzed.

We here provide evidence of how the exposure to stress and cycles of intermittent food restriction produced selective changes of eCB elements in the HY and in the CP. Further studies are needed to clarify the involvement of eCB system in regulation and development of BE disorder focusing on the role of environmental factors in the development of BE episodes and thus on the epigenetic mechanisms triggering the observed changes in genes expression.

P12.21
ANTIDEPRESSANT ACTION OF 2,5-DIMETHOXY-4-IODOAMPHETAMINE (DOI)

Kondev V
Institute of Pharmacology-Polish Academy of Sciences Poland

In recent clinical studies, administration of psychedelics has been suggested to improve symptoms in patients with depression. Psychedelics, characterized by their ability to act as full or partial agonists of serotonin (5-HT) receptors, may thus play a therapeutic role in depression and other serotonin disorders.

We used rodent behavioral models, the forced swim test and the tail suspension test, to assess the antidepressant effects of 2,5-dimethoxy-4-iodoamphetamine (DOI), a selective 5-HT2 agonist with hallucinogenic properties. Rats ($n = 10$) were injected twenty-four hours before each test. It has also been hypothesized that the antidepressant effects of psychedelics may involve interactions with other types of receptors, including glutamate receptors. We thus assessed possible interactions between DOI and metabotropic glutamate receptors (mGluR) by administering sub-effective doses of DOI with LY341495, a mGluR2/3 antagonist, twenty-four hours before each test.

DOI was shown to reduce immobility time in both the forced swim test and the tail suspension test, suggesting antidepressant action. Furthermore, the combined administration of DOI and LY341495 at sub-effective doses had no significant effect on either behavior compared to controls.

These results further confirm previous studies that hallucinogens may have therapeutic potential for depression. Additionally, our results show that the antidepressant action of DOI is independent of mGluR2/3 activation.

P12.22
THE LONG TERM BRAIN EFFECTS OF BINGING ON ALCOHOL AND MARIJUANA IN ADOLESCENT TOBACCO USERS: A BEHAVIOURAL AND NEUROCHEMICAL STUDY IN RATS "THE PACEVILLE PROJECT I"

Haywood K[1,2], Casarrubea M[3], Abela N[1], Pierucci M[1], Camilleri SM[1], Crescimanno G[3], Benigno A[3], De Deurwaerdere P[4] and Di Giovanni G[1,2]
[1]*Laboratory of Neurophysiology Department of Physiology and Biochemistry, Faculty of Medicine and Surgery, University of Malta - Msida, Malta*
[2]*School of Biosciences, Cardiff University - Cardiff, U.K.*
[3]*Department of Experimental Biomedicine and Clinical Neurosciences, Human Physiology Section "Giuseppe Pagano", University of Palermo - Palermo, Italy*
[4]*CNRS, Institut des Maladies Neurodégénératives, UMR 5293, F-33000 Bordeaux, France*

Adolescence is a critical developmental period, with respect to anatomical, neurochemical and behavioural changes. Binge consumption of alcohol and marijuana, along with smoking of tobacco, is a dangerous pattern often observed in adolescents. This study focuses on the chronic effects of binge-like exposure to alcohol and marijuana in adolescent male and female Long-Evans rats that were receiving daily doses of nicotine.

Treatments began at postnatal day 30 (P30) and lasted for 28 days. Daily intraperitoneal (i.p.) injections nicotine (1 mg/kg) were given, along with administration of IP injections of WIN55,212-2 (1.2 mg/kg) and gavage feeding of ethanol (3 g/kg) on two consecutive days a week, referred to as 'binging days' to 10 males and 10 females. The remaining 10 males and 10 females received vehicles with the same timing. Behavioural tests were conducted at P30, P60 and P90 and subsequent neurochemical analysis will be performed.

Changes in levels of anxiety were measured using the hole-board (HB) test and elevated plus maze (EPM). For a more detailed analysis on the long-term effects of the drug combination, temporal pattern (t-pattern) analysis will be conducted to detect hidden reoccurring patterns of behavioural elements otherwise difficult to observe. In addition, monoamine concentrations of selected brain regions typically involved in anxious states will be analysed using high-performance liquid chromatography coupled with electrochemical detection (HPLC-ED).

Results generated from HB and EPM analysis showed no significant effects of the drug combination, although

differences in behavioural elements between sexes were observed. With increasing age, male rats displayed decreased mean occurrences and durations of exploratory behaviours, irrespective of treatment type.

Our findings suggest that male and female rats react differently to adolescent stress induced by the treatment procedures. Due to the critical neurodevelopmental changes that take place during adolescence, and the susceptibility to long-term damage during this period, the chronic effects of heavy drinking and drug use are important to elucidate.

P12.23
SEROTONIN-2A AND -2C RECEPTOR MODULATION OF THE LATERAL HABENULA ACTIVITY IN THE CONTEXT OF NICOTINE ADDICTION: A NEUROANATOMICAL AND ELECTROPHYSIOLOGICAL STUDY

Delicata F[1], Pierucci M[1], Bombardi C[2], Benigno A[3] and Di Giovanni G[1,4]

[1]*Department of Physiology and Biochemistry, Faculty of Medicine and Surgery, University of Malta - Msida, Malta*

[2]*Dipartimento di Scienze Mediche Veterinarie, Università di Bologna - Bologna, Italia*

[3]*Department of Experimental Biomedicine and Clinical Neurosciences, Human Physiology Section "Giuseppe Pagano", University of Palermo - Palermo, Italy*

[4]*School of Biosciences, Cardiff University - Cardiff, U.K.*

The aim of this study was to investigate the role of the 5-HT_{2C} receptors (5-HT_{2C}Rs) in the modulation of the lateral habenular nucleus (LHb), under normal condition, and after acute and chronic nicotine treatment in rats. Due to the high homology between 5-HT_{2C}Rs and 5-HT_{2A}Rs, we studied both 5-HT receptors subtypes.

The expression of 5-HT_{2A}Rs and 5-HT_{2C}Rs in the LHb was investigated by immunohistochemical approach using mouse anti-5-HT_{2A}R/5-HT_{2C}R monoclonal antibodies. Immunohistochemical experiments showed a diffuse 5-HT_{2A}R and 5-HT_{2C}R immunolabelling in cell bodies and neuropil of the LHb. We show for the first time that 5-HT_{2A}Rs are expressed on LHb neurons, and are present in a similar proportion to 5-HT_{2C}Rs.

Standard single cell extracellular recordings were performed *in vivo* in anaesthetized rats. The effects of intravenous (i.v.) administration of two 5-HT^2R ligands on LHb neuronal activity were investigated: RO60-0175 (preferential 5-$HT_{2B/2C}$R agonist); and TCB-2 (potent 5-HT_{2A}R agonist). RO60-01745 (5–640 µg/kg,i.v.) induced changes in 39% of the neurons treated, which responded with either an increase (9%) or a decrease (30%) in their firing rate. The change in firing activity was dose dependent, with maximum effects elicited towards the higher doses for both excitation and inhibition ($+59 \pm 22\%$ and $-56 \pm 8\%$). Both effects were blocked by the administration of SB242084, a selective 5-HT_{2C}R antagonist. TCB-2 (5–640 µg/kg, i.v.) affected 79% of the neurons treated, which responded with either an increase (26%) or a decrease (53%) in their firing rate. The change in firing activity was dose dependant, with maximum effects elicited towards the higher doses ($+102 \pm 32\%$ and $-61 \pm 12\%$). These effects were both reversed by the administration of MDL11,939, a selective 5-HT_{2A}R antagonist. Pre-treatment with either SB242084 or MDL11,939 significantly altered the nature of LHb neuronal response to TCB-2 (5–640 µg/kg, i.v.).

Acute nicotine treatment reduced the overall firing rate of LHb neurons and increased their irregularity in firing. Chronic nicotine treatment up-regulated 5-HT_{2C}R expression. Nicotine treatments were shown to attenuate the inhibitory response to RO60-0175 administration, and the excitation response to TCB-2 administration. Our data shows that both 5-HT_{2A}Rs and 5-HT_{2C}Rs bidirectionally modulate LHb neuronal output. These findings are important for their physiological relevance and for therapeutic intervention in the cessation of nicotine abuse and drugs of addiction in general.

P12.24
ROLE OF THE KAPPA OPIOID SYSTEM ON THE FACILITATING EFFECTS OF PRENATAL ALCOHOL EXPOSURE ON LATER ALCOHOL INTAKE

Wille-Bille A, Pautassi, RM and D'Addario C

Instituto de Investigación Médica Mercedes y Martín Ferreyra (INIMEC-CONICET-UNC), Córdoba, Argentina

Several experiments indicated that moderate prenatal alcohol exposure (PEE, 1–2 g/kg, gestational days 17 to 20) induces a significant, facilitatory effect on subsequent ethanol consumption in infant or adolescent rats. This effect may be the consequence of PEE enhancing or reducing the appetitive and aversive motivational effects of ethanol, respectively. The mechanisms underlying PEE effects are, however, still elusive. The endogenous opioid system has been proposed as an important target of alcohol's actions and ethanol exposure seems to alter the developmental trajectory of opioid systems, possibly affecting the hedonic effect of ethanol. The aim of this study was to describe the effect of PEE on subsequent, voluntary alcohol consumption, and on opioid system gene expression.

Pregnant rats received daily intragastric administration of alcohol (0.0 or 2.0 g/kg). Female and male offspring were tested at infancy or adolescence. We ana-

lyzed anxiety response and ethanol-induced locomotor activity (infants and adolescents), alcohol intake (adolescents), and gene expression levels of predynorphin (PDYN) and kappa opioid receptors (KOR) in mesocorticolimbic areas of the brain (infants and adolescents).

PEE was associated with elevated anxiety response in infants and with a blunted response to the stimulant effects of ethanol during adolescence. PEE male, adolescent, rats consumed signficantly higher amounts of alcohol than control peers. Notably, several PEE-induced alterations on gene expression were observed. During infancy, PEE signficantly enhanced levels of KOR in Prefrontal Cortex and PDYN in Ventral Tegmental Area (VTA), and signficantly lowered levels of PDYN in Nucleus Accumbens. PEE adolescents exhibited higher levels of KOR in VTA, than control rats.

These results confirm that a moderate exposure to alcohol during the last days of pregnancy is a risk factor for subsequent ehancenment of alcohol intake at adolescence. The study also pinpoints alterations in behavior and gene expression that could be responsible of the facilitatory effects of PEE.

P12.25 ENDOCANNABINOID STIMULATION IN PREGNANCY: WHAT HAPPENS TO THE OFFSPRING?

Cavallaro A[1], Brancato A[1], Lavanco G[1], Castelli V[1], Plescia F[1], Melis M[2] and Cannizzaro C[1]

[1]*Department of Sciences for Health Promotion and Mother and Child Care "G. D'Alessandro" University of Palermo, Italy*

[2]*Division of Neuroscience and Clinical Pharmacology, Department of Biomedical Sciences University of Cagliari, Italy*

Prenatal exposure to cannabis is an underestimated matter that requires a specific focus in order to elucidate the negative consequences on the progeny.

The present study aims at assessing the alteration induced by the prenatal stimulation of the endocannabinoid system on behavioural reactivity, emotional memory and alcohol vulnerability in offspring rats. Pregnant dams received a stimulation of the endocannabinoid system from GD 5 to 20. The young offspring was tested from PND 25 onwards for behavioural reactivity in the Open Field and in the Elevated-Plus Maze test; and for fear-associated memory in the emotional-object recognition test. The progeny was also exposed to a Binge-drinking paradigm for assessing vulnerability to alcohol drinking. Prenatal stimulation of the endocannabinoid system increased locomotor activity in the progeny, as shown by a significant increase in total distant travelled ($p < 0.0020$) and in number of total entries ($p < 0.0051$) in Open Field and Elevated-Plus Maze, respectively, compared to controls, although it did not modify anxiety-like behaviour. When tested for the emotional memory, the endocannabinoid-stimulated rats showed impaired declarative memory associated to aversive emotional stimuli, since they did not display avoidance of a fear-associated object and showed a significant decrease in the Emotional-Object Avoidance Index ($p = 0.0255$) and in the emotional zone-related Difference Score ON-BSL ($p < 0.05$), with respect to controls. In the same rats, increased vulnerability to alcohol binge drinking was recorded: the analysis of binge-drinking pattern indicated increased alcohol-intake ($p < 0.001$) and preference ($p < 0.001$) when compared to controls.

In conclusion, this research highlights that prenatal exposure to cannabinoids for medical or recreational purposes, does induce complex disarrangement in the brain progeny, that arises during adolescence and involve emotionally salient memories and vulnerability to drug of abuse.

P12.26 *IN VIVO* ASSESSMENT OF SEROTONIN TRANSPORTER DENSITY IN THE MOUSE BRAIN: A PET/MR APPROACH

Reisinger SN[1], Wanek T[2], Langer O[2,3] and Pollak DD[1*]

[1]*Department of Neurophysiology and Neuropharmacology, Center for Physiology and Pharmacology, Medical University of Vienna, Austria*

[2]*Biomedical Systems, AIT Austrian Institute of Technology GmbH, Austria*

[3]*Department of Clinical Pharmacology, Medical University of Vienna, Vienna, Austria*

The serotonin transporter (SERT) plays an important role in the regulation of serotonergic neurotransmission, and its aberrant expression has been linked to psychiatric conditions, including major depressive disorder (MDD). While SERT density has previously been proven to be amenable to in-vivo quantitative evaluation by positron emission tomography (PET) in humans, this approach is only beginning to be developed for rodents. Considering the important role of rodent models as experimental system for the exploration of the pathophysiology of MDD, the aim of this study was to evaluate the feasibility of using small-animal PET employing [^{11}C]DASB ([^{11}C]-3-amino-4-(2-dimethylaminomethyl-phenylsulfanyl)-benzonitrile) as a radiotracer to measure relative SERT density in designated areas of the mouse brain.

To this end, wild-type, heterozygous and homozygous SERT knockout mice were used in a PET/MR approach. Anatomical MR images of the brain were acquired before placing the mice in a small-animal PET scanner un-

der administration of [^{11}C]DASB for dynamic PET imaging. A simplified reference tissue model (SRTM) using the cerebellum as reference region was used to calculate the binding potential (BP_{ND}) as a quantitative parameter of SERT density. PET data were complemented and validated by ex-vivo measurements of SERT protein expression by Western Blot and respective correlations between *in vivo* and *ex-vivo* results were calculated.

The obtained data firstly demonstrate small-animal PET in mice as reliable and useful tool for the investigation of SERT protein density in the intact animal and suggest its application for the exploration of dynamic changes in SERT levels in animal models of MDD. The utility of this approach is currently being tested in a longitudinal study aiming to measure relative SERT density *in vivo* at baseline and in response to chronic stress exposure.

P12.27
SCHIZOPHRENIA AND CANNABINOID RECEPTOR TYPE1 GENE REGULATION: A PRECLINICAL AND CLINICAL STUDY

Di Bartolomeo M[1], Micale V[2,3], Stark T[4], Pucci M[1], Sulcova A[2], Palazzo M[5], Babinska Z[4], Cremaschi L[6], Drago F[3], Altamura AC[6], Maccarrone M[7,8], Dell'Osso B[6,9] and D'Addario C[1,10]

[1]*Faculty of Bioscience and Technology for Food, Agriculture and Environment, University of Teramo, Italy*
[2]*CEITEC/Masaryk University, Brno, Czech Republic*
[3]*Department of Biomedical and Biotechnological Science, Section of Pharmacology, University of Catania, Catania, Italy*
[4]*Masaryk University, Faculty of Medicine, Department of Pharmacology, Brno, Czech Republic*
[5]*Centro Sant'Ambrogio, Ordine Ospedaliero San Giovanni di Dio-Fatebenefratelli, Cernusco sul Naviglio, Italy*
[6]*Department of Neuroscience, University of Milan, Fondazione IRCCS Ca' Granda Ospedale Maggiore Policlinico, Milano, Italy*
[7]*Campus Bio-Medico University of Rome, Department of Medicine, Rome, Italy*
[8]*European Center for Brain Research IRCCS Santa Lucia Foundation, Rome, Italy*
[9]*Department of Psychiatry and Behavioral Sciences, Bipolar Disorders Clinic, Stanford University, CA, USA*
[10]*Dept. Clinical Neuroscience, Karolinska Institutet, Stockholm, Sweden*

Schizophrenia (SZ) is a chronic debilitating neuropsychiatric disorder, representing the eighth cause of disability in adolescents and adults. To date the causes and the molecular basis of schizophrenia are still one of the greatest challenges in psychiatry. The Endocannabinoid System (ECS) is highly represented in brain areas implicated in processing of emotionally information as well as learning and memory, such as the prefrontal cortex and several preclinical and clinical data support the hypothesis that disturbance of the ECS can have a role in the pathophysiology of schizophrenia. In this study we evaluated whether regulation of ECS genes expression might to be involved in the development and progression of SZ in a gestational rat model (prenatal administration of the mitotoxin methylazoxymethanol acetate (MAM)) as well as in human subjects.

Genomic DNA and total RNA have been isolated from rats prefrontal cortex as well as from peripheral blood mononuclear cells (PBMCs) of a cohort of human SZ and controls subjects. Quantitative Real-Time RT-PCR and Pyrosequencing have been used, respectively, to quantitatively assess the state of ECS genes expression and DNA methylation at gene promoters.

We here report a selective increase in Cannabinoid receptor type 1 gene (CNR1) expression in the prefrontal cortex of MAM rats (2.36 ± 0.34) when compared to control rats (1.14 ± 0.26, $p < 0.01$), as well as in the PBMCs of SZ patients ($p < 0.05$). Consistently a significant reduction in DNA methylation at gene promoter was observed in both MAM rats (MAM: $2.65\% \pm 0.55$; Controls: $4.77\% \pm 0.72$; $p < 0.05$) and human patients ($p < 0.01$). Overall, the present findings provide new insights into the control of pathological gene expression in SZ and suggest CNR1 gene regulation via epigenetic mechanisms as a new tool for the development of new treatment strategies in SZ.

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P13.1
MAGNETIC RESONANCE IMAGING CHARACTERIZATION OF EARLY PD DEVELOPMENT

Rosa I[1], Di Censo D[1], Ranieri B[1,2], Galante A[1,2,3], Scarnati E[4], Florio TM[1,2] and Alecci M[1,2,3]

[1]*Department of Life, Health and Enviromental Sciences, University of L'Aquila, L'Aquila, Italy*
[2]*Laboratori Nazionali del Gran Sasso, Istituto Nazionale di Fisica Nucleare, L'Aquila, Italy*
[3]*SPIN-CNR Institute, CNR, L'Aquila, Italy*
[4]*Department of Biotechnological and Applied Clinical Sciences, University of L'Aquila, L'Aquila, Italy*

Imaging biomarkers are needed to characterize PD development as soon as possible during the long lasting asymptomatic evolution of the disease, as well as to monitor the response to therapeutic interventions.

Magnetic Resonance Imaging (MRI) plays an important role in characterizing the preclinical evolution of PD showing that different striatal areas can influence motor symptomatology. It has been recently demonstrated

that MRI is able to reveal structural changes in brain grey and white matter during learning. In a previous study, we were able to reveal the presence of striatal structural changes and correlate them with task switching inabilities induced by 6-OHDA unilateral injury of the nigrostriatal pathway.

In the present study, on the basis of the behavioural effects of apomorphine treatment during the early progression of an intranigral 6-OHDA rat model, the structural properties of the Striatum were investigated by high resolution, *ex vivo*, MRI quantitative techniques and relaxometry (T1, T2, T2*). Neither the intact, nor the sham-lesioned whole brains showed interhemispheric differentiation of the main relaxometric indices. On the contrary, all lesioned brains revealed a variation in T1 and T2 values in the ipsilateral Striatum with respect to the contralateral one. The high resolution T2*-weighted images revealead a complex pattern of grey/white matter ratio, thus indicating a change in the texture of the Striatum. At present, major MRI abnormalities seen in Parkinsonism are tissue atrophy, measured in T1-weighted images, and changes of tissue signal amplitude, seen in T2-weighted images. Here we have reported a preliminary regional and temporal correlation study between behavioural and structural changes in a rat model of PD.

P13.2 DIFFERENTIAL RESPONSIVENESS OF NUCLEUS ACCUMBENS DOPAMINE TO STIMULI INSTRUMENTALLY CONDITIONED TO DRUG REINFORCERS

Sil A[1], Lecca D[1], Bassareo V[1], Pisanu A[2], Frau R[1], Scifo A[1] and Di Chiara G[1]
[1]*Department of Biomedical Sciences, University of Cagliari, Via Ospedale, 72, Cagliari 09124, Italy*
[2]*Institute of Neuroscience, National Research Council, Italy*

Nose-poking (NP) and lever-pressing (LP) represent two different response modalities which have been utilized in the self-administration (SA) paradigm. NP is part of a rodent's natural exploratory repertoire, whereas LP requires the animal to learn the action of pressing a lever in order to obtain a reward. The objective of these experiments were to study differences in the mesolimbic dopaminergic responsiveness induced by heroin self-administration (SA) using LP and NP as operant responses.

Male Sprague-Dawley rats were trained for 10 days on an FR1 schedule to acquire heroin SA (0.05 mg/kg) using NP or LP. After acquisition of SA behaviour, microdialysis was carried during a heroin SA session on an FR1 schedule to measure dialysate dopamine(DA) in the nucleus accumbens (NAc) shell and core of animals. Next day, the animals underwent an extinction session. On the third day, a dose of 0.025 mg/kg heroin was utilised while another group underwent a passive administration of heroin.

Results show that during heroin SA, dialysate DA preferentially increased in the shell only in the NP group, while DA increased both in the shell and core in the LP group. During the extinction DA did not change from basal values in both groups. DA was observed to increase in the core of both groups during passive non-contingent presentation of heroin. When the heroin dose was halved, the NP group showed even more pronounced differences in dialysate DA released in the shell and core while the LP group showed an almost equal release of dialysate DA in both.

These results add to the growing body of evidence about the differential involvement of the NAc shell and core in different aspects of reinforcement and incentive learning. Further, they show that the specific operant response utilised for modelling SA behaviour is able to determine the pattern of activation of DA transmission in the NAc core and shell.

P13.3 CHRONIC TREATMENT WITH 1-METHYL-1,2,3,4-TETRAHYDROISOQUINOLINE PROTECTS LACTACYSTIN-INDUCED DECREASE OF DOPAMINE RELEASE

Wąsik A, Romańska I and Antkiewicz-Michaluk L
Institute of Pharmacology, Polish Academy of Sciences, Department of the Neurochemistry, Smętna 12, 31-343 Kraków, Poland

The ubiquitin-proteasome system (UPS) is one of the important mechanisms for protein degradation. The UPS irreversible inhibitor, lactacystin induced the loss of dopaminergic neurons and increased the formation of cytoplasmatic inclusions. The UPS dysfunction plays significant role in the pathogenesis of Parkinson's disease (PD).

In our *in vivo* study we investigated the impact of acute or chronic treatment with 1-methyl-1,2,3,4-tetrahydroisoquinoline (1MeTIQ; 50 mg/kg i.p.) an endogenous amine with neuroprotective and MAO-inhibiting properties on the decrease in the striatal dopamine release evoked by lactacystin (5 µg/2 µl) administered unilaterally to the substantia nigra. Rats received 1MeTIQ once or chronic during 7 days, and *in vivo* microdialysis study was carried out 7 days after lactacystin lesion. The dopamine and its extraneuronal metabolite, 3-MT was assayed in dialysates using HPLC with ED. Moreover, the locomotor activity of rat was measured.

The behavioral test showed that unilateral lactacystin lesion of substantia nigra did not change the rats locomotor activity however chronic treatment with 1MeTIQ combined with lesion decreased the exploratory activity. The biochemical *in vivo* study indicated that unilateral lesion with lactacystin significantly (of about 50%; $p < 0.01$) reduced the dopamine release into the extracellular space. Both, acute and chronic treatment of 1MeTIQ completely antagonized lactacystin-induced impairment of dopamine system activity, and dopamine concentration was significantly elevated up to the control value. Simultaneously, the level of extraneuronal dopamine metabolite, 3-MT was strongly increased.

1MeTIQ has shown a clear neuroprotective activity in the lactacystin model of Parkinson's disease. The mechanism responsible for these effects may be connected with properties of 1MeTIQ to scavenge a free radicals production, and possible reduction of lactacystin uptake as DAT inhibitor into the dopamine neuron in the brain.

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P13.4
A NOVEL THERAPEUTIC STRATEGY FOR THE PREVENTION OF THE ONSET OF DYSKINESIA IN THE THERAPY OF PARKINSON'S DISEASE

Annalisa Pinna[1], Giulia Costa[2], Liliana Contu[2], Marcello Serra[2], Nicola Simola[2], Manolo Carta[2], Micaela Morelli[1,2]

[1]*National Research Council of Italy, Neuroscience Institute – Cagliari (Italy)*

[2]*Department of Biomedical Sciences, University of Cagliari, Cagliari, Italy*

The mixed serotonin 5-$HT_{1A/1B}$ receptor agonist eltoprazine suppressed dyskinetic-like behavior in animal models of Parkinson's disease (PD), but simultaneously reduced L-dopa-induced motility. Moreover, adenosine A2A receptor antagonists, as preladenant, significantly increase L-dopa efficacy in PD without exacerbating dyskinetic-like behavior. Our previous report demonstrated that combination of eltoprazine, with preladenat produces prevention and reduction of L-dopa-induced dyskinesia, without impairing the efficacy of L-dopa in relieving motor symptoms.

On this basis, we hypothesize that the early combined administration of eltoprazine and preladenant may produce prevention of the onset of L-dopa-induced-dyskinesia in a rodent model of PD.

Unilateral 6-OHDA-lesioned L-dopa-non primed rats, were treated for two weeks with eltoprazine (0.6 mg/kg) and/or preladenant (0.3 mg/kg), alone or in combination with L-dopa (4 mg/kg), and abnormal involuntary movements (AIMs) as index of dyskinesia, were evaluated. Four days after the last administrations all rats were treated with L-dopa. Moreover, induction of immediate-early gene *zif-268* (an index of long-term changes correlated with dyskinesia), and microglia and astroglia markers (indexes of neuroinflammation) were evaluated.

Results show that combined administration of L-dopa plus eltoprazine plus preladenant significantly prevented and delayed the onset of dyskinetic-like behaviors induced by L-dopa.

Preliminar results showed that *zif-268* was increased in striatum of rats treated with L-dopa and L-dopa plus preladenant compared with vehicle. In contrast, rats treated with eltoprazine (with or without preladenant) had lower *zif-268* activation after treatment in L-dopa-non-primed rats.

Results suggest that combination of L-dopa with eltoprazine and preladenant may be a promising therapeutic strategy for treating motor symptoms, delaying, at the same time, the onset of dyskinesia in PD.

P13.5
THE ROLE OF METALLOPROTEINASES-2 AND -9 AND THEIR INHIBITORS IN NEUROTOXICITY OF FLUORINE

Gutowska I[1], Baranowska-Bosiacka I[2], Łukomska A[1], Tarnowski M[3], Pilutin A[4], Dec K[1], Goschorska M[2] and Chlubek D[2]

[1]*Department of Biochemistry and Human Nutrition*

[2]*Department of Biochemistry*

[3]*Department of Physiology*

[4]*Department of Histology and Embryology, Pomeranian Medical University in Szczecin, Poland*

Fluorine is a strong neurotoxin which can decrease the intelligence quotient and cause problems with learning and concentration. The Extracellular Matrix (ECM) of the central nervous system serves as the environment for neurons and glial cells, and at the same time, it plays the role of a modifier of these cells. Changes in the structure and the functioning of synapses are caused by ECM enzymes. These enzymes, especially matrix metalloproteinases (MMPs), accompany both physiological processes, such as learning or memorizing, and pathological processes. Metalloproteinases 9 and 2 (MMP-9 and MMP-2) and the inhibitors of metalloproteinases-3 and -2 (TIMP-3 and TIMP-2) seem to be particularly interesting. There is no data regarding the influence of fluorine on the expression of these enzymes and their inhibitors in the brain.

In the research, the rats were exposed to sodium fluoride (50 mg/L) already in the prenatal period until they

reached the age of three months. After this time, the hippocampus, prefrontal cortex, cerebellum, and striatum were collected. In all of the aforementioned structures, the expression of proteins MMP-9, MMP-2, TIMP-3 and TIMP-2 was carried out by means of ELISA, gene expression by RT real time PCR and immunolocalization by immunohistochemistry and microscopic visualization.

On the basis of the results, it can be concluded that fluorine influences the expression of MMP-9, MMP-2, TIMP-3 and TIMP-2. In the study group, a statistically significant expression of MMP-2 was observed in the prefrontal cortex, striatum, and cerebellum, and a decrease in the expression of MMP-9 was noted in the prefrontal cortex and cerebellum in relation to the control group. We also saw the difference between TIMP-3 and TIMP-2 levels in the study group compared to control.

Our research suggests that changes in the expression of metalloproteinases and their inhibitors in the brain, caused by fluorine, could be an important factor of neurotoxicity of fluorine. The disorders of neuroplasticity processes can be considered as a biochemical basis for the decrease in the intelligence quotient caused by fluorine.

P13.6
THALIDOMIDE ATTENUATES L-DOPA-INDUCED DYSKINETIC RESPONSES AND NEUROINFLAMMATION IN THE DENERVATED STRIATUM OF EMIPARKINSONIAN RATS

Boi L[1], Mulas G[1], Spiga S[2], Pisanu A[3] and Fenu S1and Carta AR[1]

[1]*Department of Biomedical Sciences, University of Cagliari, Cagliari, Italy*

[2]*Department of Life and Environmental Sciences, Cagliari, Italy*

[3]*National Research Council, Institute of Neuroscience, Cagliari, Italy*

L-DOPA therapy represents the main treatment for Parkinson's Disease (PD), however long-term administration results in treatment-related motor complications named L-DOPA-induced dyskinesia (LID). Preclinical studies have suggested a role of inflammatory responses in the onset ofAbnormal Involuntary Movement (AIMs), a model of LID in the 6-OHDA rat model of PD. In this model, a L-DOPA chronic treatment eliciting AIMs is associated with astrocytosis, microgliosis and increased levels of Tumor Necrosis Factor-α (TNF-α) within OX-42-positive cells.

Thalidomide is a powerful anti-inflammatory and immunomodulatory drug through inhibition of TNF-α and suppression of Nuclear Factor kappa B. We investigated whether thalidomide may prevent the onset of AIMs and reduce L-DOPA-induced inflammatory responses in the striatum of 6-OHDA-lesioned rats.

6-OHDA-lesioned rats received ten days repeated treatment with L-DOPA+Benserazide (6 mg/Kg), Thalidomide (70 mg/Kg), or Thalidomide thirty minutes before L-DOPA+Benserazide administration. Limb, axial AIMs and contralateral turning response were evaluated daily, TNF-α immunoreactivity (IR) was quantified within OX-42-positive microglia in the denervated dorsolateral striatum one hour after the last L-DOPA administration.

Rats receiving repeated L-DOPA displayed AIMs and controlateral turning behavior, which progressively increased during treatment. Moreover, L-DOPA-treated rats displayed increased OX-42 IR and TNF-α/OX-42 colocalization in the dorsolateral striatum as compared to vehicle-treated rats. In contrast, treatment with Thalidomide + L-DOPA induced limb and axial AIMs of lower intensity than L-DOPA. In the striatum, thalidomide inhibited L-DOPA-induced increases of OX-42 and TNF-α IR.

Our results show that thalidomide reduced L-DOPA-induced dyskinetic responses as well as microgliosis and TNF-α production, suggesting that targeting the neuroinflammatory response may alleviate the development of L-DOPA-induced dyskinesia in PD.

P13.7
NEUROPROTECTION OFFERS BY AN ENDOGENOUS AMINE, 1-METHYL-1,2,3,4-TETRAHYDROISOQUINOLINE IN HEMIPARKINSONIAN RATS: THE BIOCHEMICAL AND MOLECULAR STUDY

Antkiewicz-Michaluk L, Wąsik A, Romańska I and Michaluk J

Institute of Pharmacology Polish Academy of Sciences, Department of the Neurochemistry, Smętna 12, 31-343 Kraków, Poland.

There is a growing body of evidence that impairment of the ubiquitin-proteasome system (UPS) in the substantia nigra (SN) plays an important role in the pathogenesis of Parkinson's disease (PD). The loss of dopamine (DA) cells in the substantia nigra pars compacta and consequently further more in the extrapyramidal nigrostriatal DA neurons are the characteristic pathological hallmark of PD. The aim of the present study was to investigate the effect of acute and multiple (7 days) administration of 1-methyl-1,2,3,4-tetrahydroisoquinoline (1MeTIQ; 50 mg/kg i.p.), an endogenous amine with neuroprotective and MAO-inhibiting properties on the biochemical and molecular markers of dopamine neurons

injury evoked by UPS inhibitor, lactacystin (5 µg/2 µl) administered unilaterally to rat SN. The DA and its metabolites (DOPAC, 3-MT, HVA) was assayed in the ipsi- and contralateral striatum using HPLC with ED. Additionally, the level of tyrosine hydroxylase (TH) in the SN at using Western blot method and the amount of Bcl-2 protein (ELISA) in the hippocampus was also determined. The biochemical *ex vivo* study carried out 7 days after lactacystin lesion have demonstrated a significant reduction the concentration of DA (of about 65%; $p < 0.01$) and its metabolites (from 35% to 50%; $p < 0.05$) in the ipsilateral striatum. Similarly, the level of TH and the amount of antiapoptotic protein Bcl-2 was essentially decreased (of about 25% and 50%, $p < 0.05$; respectively) in the investigated brain structures. Both, acute or multiple administration of 1MeTIQ completely antagonized the lactacystin-induced impairment of the DA system activity. The level of DA as well as the concentration of TH and Bcl-2 protein elevated up to the control values in the joint groups. The present results add a new value to the study of 1MeTIQ-produced neuroprotection of the lactacystin-induced neurodegeneration as a valuable animal model of PD.

The study was financially supported through a funds of KNOW (The National Scientific Leading Center) from the Institute of Pharmacology, Polish Academy of Sciences, Krakow, Poland.

P13.8
INTERMITTENT HYPOXIA INCREASES TAU PHOSPHORYLATION VIA BIOLOGICAL PROCESSES COMMON TO AGING: POTENTIAL LINK BETWEEN SLEEP-DISORDERED BREATHING AND ALZHEIMER DISEASE

Yagishita S
Department of Peripheral Nervous System Research, National Institute of Neuroscience, National Center of Neurology and Psychiatry, Japan

Alzheimer disease (AD). Patients with SDB often have cognitive impairments, which are associated with intermittent hypoxia. Thus, intermittent hypoxia treatment (IHT) has been used as an experimental animal model for SDB. In this study, we aimed to elucidate molecular mechanisms linking SDB and AD.

Mice underwent IHT protocols, and their hippocampal samples were subjected to gene ontology (GO)-based microarray analyses and biochemical analyses. The mice were also subjected to behavioral analyses.

GO-based microarray analyses including various public data revealed that IHT and aging shared alterations in some common GO, which were also observed with kainic acid treatment, Dicer ablation, or moderate glutamate excess. Further *in silico* analyses indicated that IHT led to alterations in the imbalance of kinases and/or phosphatases and glutamatergic synapse. Thus, we performed *in vivo* analyses regarding tau, which has been focused in AD research, and glutamatergic synapse. As the results, we found that IHT significantly increased phosphorylated tau, and reduced proteins relevant to glutamatergic synapses. In addition, IHT increased phosphorylated p70 S6 kinase, indicating involvement of the mammalian target of rapamycin signaling pathway. Furthermore, IHT mice demonstrated hyperactivity in Y-maze tests, which was also observed in AD models.

These results may explain the potential link between SDB and AD. Aging is a major risk factor for AD, therefore, IHT is a novel model for investigating the pathological processes contributing to AD onset.

P13.9
SEX DIFFERENCES ON SEQUENTIAL ACTIVATION OF MICROGLIA AND ASTROCYTE FOLLOWING POSTNATAL SYSTEMIC IMMUNE CHALLENGE

Berkiks I, Mesfioui A and Elhessni A
Laboratory of Genetic, Neuroendocrinology and Biotechnology -Faculty of Sciences, Ibn Tofail University, Kenitra, Morocco

Early immune challenges induce long-lasting brain developmental and behavioral impairments and increase the risk of diseases later in adulthood. The activation of the immune system results in the release of proinflammatory cytokines, such as interleukin-1β (IL-1β), tumor necrosis factor α (TNF-α) and IL-6. High levels of these cytokines during development are associated with low resilience to diseases in adulthood. The recent study demonstrated that the glial cells has a sequential activation, for example, the height level of cytokines production during the inflammation activate the microglia fist which leads to activate the astroglia after.

The aim of this study is: To compare the behavioral responses of the glial cells after different postnatal LPS challenge times in both of sexes. By measuring the cytokines, and oxidative stress level.

P13.10
REAL-TIME SEIZURE ONSET DETECTION USING WAVELETS AND COMMON SPATIAL PATTERN WITH EMPIRICAL MODE AND GRAPH SPECTRAL DECOMPOSITIONs

Audu EE[1], Garg L[1], Falzon O[2] and Di Giovanni G[3]
[1]*Department of Computer Information Systems, University of Malta, Msida, Malta*
[2]*Centre for Biomedical Cybernetics, University of*

Malta, Msida, Malta
[3]*Department of Physiology and Biochemestry, University of Malta, Msida, Malta*

Epilepsy, clinically, is a medical condition associated with neurological abnormality as a result of irregular electrical discharge of a group of neurons. This aberration negatively distorts normal brain function, and the extend can vary from brief relapse in state of attention to loss of consciousness. Epilepsy is associated with recurrent, and people living with the disorder are prone to emergency room cases and social disqualification due to continuous cycle of stigmatization. One out of every three of the 50 Million patients are unresponsive to surgery and anti-convulsion treatments. Lack of effective drug therapy that can suppress seizures in epileptic patients can negatively impact on their quality of life, economic, psychological and social wellbeing.

To augment the clinical management of epilepsy, the application of signal processing and machine learning techniques have opened a new frontier in automatic seizure detection by analyzing and evaluating electrical signals captured from the brain. The fundamental problems in developing such algorithms are: How to encode knowledge domain algorithmically in the system to perform in comparison to human expert and the ability of the system to adapt to variability between different seizures and patients.

The main focus of this work is to build a robust feature extraction method and machine learning classification. A key step in the program development is decomposing EEG into main features, which involves identifying notable patterns that can distinguish between seizure and non-seizure states using mathematical and computing models. We propose the use of Wavelets and common spatial patterns (CSP) with empirical mode decomposition and graph spectral decomposition methods to describe abnormal EEG in terms of spectral, temporal and spatial information. CSP with spectral graph decomposition method is use in the preprocessing stage where the derived spatial coefficients optimally maximize variance (or band power) of seizure state class while, simultaneously, minimizing the variance of normal EEG background information. We explore graphic theoretic approach to CSP provided a link through which abnormal EEG components can be treated as a graph partitioning problem.

The features extracted are treated as binary classification problem where data are classified as either seizure, and non-seizure using machine learning approach.

P13.11
INVESTIGATING THE EFFECTS OF THE DESIGN OF TES ELECTRODES ON SKIN IMPEDANCE

Falzon A, **Grech SK**, Russo E and Trevisan AA
AAT Medical Ltd, San Gwann, Malta

Transcranial electrical stimulation (tES) is applied in a range of biomedical applications, allowing for direct neurofeedback through the application of weak electrical current through the scalp using dedicated electrodes.

Three different categories of electrodes are mainly used in tES applications; self-adhesive, rubber carbon pads, and sponge electrodes. All three present individual benefits and can be successfully applied to tES applications. It has been widely found in literature that sponge electrodes are however best suited to current stimulation applications, primarily due to their efficiency over thick hair. This is a direct consequence of the added conductivity provided by the saline solution applied to the sponge material, which seeps through to the scalp, thereby providing direct and contiguous electrical contact.

The purpose of this study is to investigate the effect of different tES electrode designs on current stimulation. Experiments were conducted using an HD-tES device and electrodes currently being developed in-house. Electrodes and their sponge material have been designed to allow easy replacement onto a dedicated head-cap, whose design is based on the standard 10-10 EEG electrode layout, which ensures appropriate montage positioning in tES stimulation protocols.

Initial tests were conducted to determine the optimum size and amount of saline required for both square and circular sponge electrodes for the application of HD-tES, versus impedance and comfort, whilst maintaining a constant sponge thickness. Further tests were conducted to investigate the difference in impedance on the skin between square and circular electrodes in different scenarios involving the presence of hair and at different levels of skin preparedness. A control was set up using standard wet electrodes placed on the skin following preparation, and ANOVA analyses were conducted to assess the performances of the different electrode designs.

P13.12
THE ROLE OF DIFFERENT THALAMO-CORTICAL NETWORKS IN THE GENERATION OF SLEEP SPINDLES IN EPILEPTIC PATIENTS

Hajnal B[1,2], Ujma PP[3], Bódizs R[3,4], Tóth E[5], Erőss L[6], Ulbert I[7] and Fabó D[1]
[1]*Epilepsy Centrum, Dept. of Neurology, National Institute of Clinical Neurosciences, Budapest, Hungary*
[2]*Semmelweis University, School of PhD studies, Budapest, Hungary*
[3]*Semmelweis University, Institute of Behavioral Science, Budapest, Hungary*
[4]*Department of General Psychology, Pázmány Péter*

Catholic University, Budapest, Hungary
[5]*Faculty of Information Technology and Bionics, Pázmány Péter Catholic University, Budapest, Hungary*
[6]*Dept. of Funct. Neurosurgery, National Institute of Clinical Neurosciences, Budapest, Hungary*
[7]*Dept. of Comparative Psychophysiology, Inst. for Psychology, Hungarian Academy of Sciences, Budapest, Hungary*

Sleep spindles are generated in thalamocortical networks. Thalamocortical projections include core projections to cortical layer IV and matrix projections to layer I-II. It has been proposed that sleep spindles can be generated in either of these networks.

We used physiological data from 5 human patients undergoing presurgical electrophysiological monitoring, including laminar microelectrodes with post-operative histological reconstruction of the electrode tracks, to test this hypothesis. Sleep spindles were detected automatically in electrocorticography channels and detection-triggered laminar microelectrode recordings were analyzed. Results indicate highly heterogeneous intracortical local field potentials during sleep spindles, and maximum current source density in layer I, II and IV. Current source density in superficial layers and layer IV is moderately correlated ($r \approx 0.4$), and the ratio of the two is normally distributed with a mean of approximately 1. These results were similar for slow and fast spindles as well as localized and global spindles.

The results confirm that both core and matrix thalamocortical projections contribute to spindle generation, but most spindles appear to be generated by a combination of the two and only a small minority by only one or the other.

P13.13
NEUROBEHAVIORAL EFFECTS OF NEONATAL RETICULAR THALAMIC NUCLEUS LESION

El Boukhari H, Ouhaz Z, Ba M'hamed S and Bennis M
Laboratory of Pharmacology, Neurobiology and Behavior (URAC-37), Cadi Ayyad University, Marrakech, Morocco

In all mammals, the thalamic reticular nucleus (TRN) occupies a strategic position lying within the fiber bundle that interconnects the dorsal thalamus and the telencephalon. The TRN is a structure that is solely composed of GABAergic inhibitory neurons, and as part of the thalamus, it receives input from the cortex and other thalamic nuclei and provides major inhibitory input to each thalamic nucleus, particularly the mediodorsal nucleus (MD). As the MD is important for supporting optimal cortico-thalamo-cortical interactions from very early on during brain maturation, previous studies of our team have shown that early insult of this nucleus induced functional and structural abnormalities in the cerebral cortex; so, what about the early lesion of the NRT on the development of the MD and the cortex? Our study assessed whether the early postnatal lesion of the RTN, reciprocally interconnected to mediodorsal thalamus, causes disruption of behavior and cognition in young adult rats.

Rat pups (postnatal day 4) were randomized in 3 groups: the first group received a bilateral electrolytic lesion of RTN, the second corresponding to RTN-sham-lesion group, and the third as a classical control group. After seven weeks, all rats were tested with the following several behavioral and cognitive paradigms, and then perfused for histological study and immunohistochemistry of glutamate.

RTN lesioned rats presented deficits in shifting capabilities and acquiring new strategies, significant hypoactivity, increasing in anxiety-like behavior and disruption of the recognition memory compared to RTN-sham-lesion and control rats. In addition, histological study showed that lesioned animals have reduced the volume of mediodorsal thalamus, and decreased number of positive glutamate cells in the PFC as well as the basolateral amygdala complex.

The different behavioral and histological alterations reported in our study suggest that early damage of the anterior part of the RTN leads to alterations may control the development of the mediodorsal thalamus-prefrontal cortex pathway.

P13.14
CYTOCHROME P450 INDUCTION IN SH-SY5Y CELLS AND ITS PROTECTIVE ROLE

Fernández-Abascal J and Valoti M
Department of Life Sciences, University of Siena, Siena, Italy

Cytochrome P450 (CYP) is one of the most important metabolic system for exogenous compounds. This isoenzyme family promote a deep systemic clearance of almost all xenobiotics, but in particular circumstances it can also lead towards cellular injury by producing toxic metabolites. In CNS it is present at different concentrations in different brain areas and can play an important role in both therapeutic effects or in toxic activation of drugs, however the effective role of CYP in the detoxification/toxic effects in brain is still under debate. In order to clarify its function, we have setup an *in vitro* model of CYPs modulation using human neuroblastoma SH-SY5Y cells.

Cells were treated with different well known liver-CYP inducers: beta-naphtoflavone (BNF), cyclophos-

phamide (CPA), and ethanol (EtOH). The expression of various CYP isoenzymes (2D, 2E1, 1A1 and 3A4) was evaluated by qRT-PCR and western blot (WB) analysis. Moreover, the CYPs cell localization was studied by confocal imaging. Finally, the toxic effect of MPP^+ was studied by Annexin V/ PI FACS analysis in control and CYP-induced SH-SY5Y cells.

The qRT-PCR and WB revealed that the treatment with BNF promoted a 1.5-fold increase of CYP2E1 and a 1.2-fold increase of CYP2D6 compared to control cells. CPA treatment increased the expression of CYP2D6 nearly 2-fold but it didn't change the expression of CYP2E1; while EtOH treatment did not increase the expression of CYP2D6, but increased about 1.5-fold the expression of CYP2E1. Confocal image analysis showed that CYP2D6 is localised in mitochondria while the other isoforms were mostly expressed in endoplasmic reticulum. Moreover, Annexin V/PI FACS analysis revealed that the treatment with BNF promoted a decrease of the toxic effect caused by MPP^+.

These data suggest that CYP can be inducible in SH-SY5Y cells and its increase can protect neurons from toxic insult promoted by MPP^+.

P13.15
CONTRIBUTION OF SPINAL 5-HT_{5A} RECEPTORS IN THE ANTINOCICEPTIVE EFFECTS OF SYSTEMICALLY ADMINISTERED CANNABINOID AGONIST WIN 55,212-2 AND MORPHINE

Aksu AG, Gunduz O and Ulugol A
Trakya University, Faculty of Medicine, Department of Medical Pharmacology, Edirne, Turkey

The antinociceptive effects of cannabinoids and opioids have been known for centuries. Serotonin and its receptors are also known to play important roles in nociception. However, contribution of spinal 5-HT_{5A} receptors in antinociceptive effects of cannabinoids and opioids has not been studied. We conducted this study to clarify mechanisms of actions of the antinociceptive effects of cannabinoids and opioids.

Hot-plate and tail-flick tests were used to assess the antinociceptive activity in Balb/c mice.

WIN 55,212-2, a cannabinoid agonist, and morphine exerted significant antinociceptive effects at 1, 3 and 10 mg/ kg doses in both hot plate and tail flick tests. Then, we administered the selective 5-HT_{5A} receptor antagonist SB-699551 (10 nmol/mouse) intrathecally 10 minutes before the agonists. SB-699551 significantly reduced the antinociceptive effect of both WIN 55,212-2 and morphine. In the rotarod test, WIN 55,212-2 disrupted the motor coordination at the dose of 10 mg/kg, while morphine did not affect this function at any doses.

Our findings show that spinal 5-HT_{5A} receptors are involved in the antinociceptive effects of WIN 55,212-2 and morphine.

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P13.16
NEUROINFLAMMATORY EFFECTS AND BEHAVIORAL CORRELATES AFTER REPEATED EXPOSURE TO THE SYNTHETIC CANNABINOID JWH-018

Pintori N[1], Simola N[1], Fattore L[2], Scherma M[1], Fadda P[1], Castelli MP[1] and De Luca MA[1]
[1]*Department of Biomedical Sciences, University of Cagliari, Italy*
[2]*Institute of Neuroscience-Cagliari, National Research Council, Cagliari, Italy*

The synthetic cannabinoid (SC) 1-pentyl-3-(1-naphthoyl)-indole (JWH-018) has been detected in several samples of a smokable herbal mixture termed Spice/K2 drugs, that are currently marketed as legal alternatives to *Cannabis*. Its use represents a growing public health worldwide. JWH-018 is a CB1/CB2 receptor agonist with higher affinity than Δ9-THC, the active ingredient of marijuana. JWH-018 shares with Δ9-THC CB1-dependent reinforcing and DA stimulant actions displaying a preferential effect on the NAc shell at the dose of 0.25 mg/kg ip. Despite the increasing popularity of Spice drugs, the effects of their chronic use are unknown. Recently, an *in vitro* study showed that SCs induce cytotoxicity in forebrain neuronal cultures in a concentration-dependent manner. However, modulation of the endocannabinoid system has been associated with both neurotoxic and neuroprotective effects.

In the present study, we evaluated the neuroinflammatory and neurodegenerative effects induced by a chronic treatment of JWH-018 in DAergic brain regions involved in emotional and cognitive processing.

To this end, rats were administered once a day for 14 consecutive days with JWH-018 (0.25 mg/kg i.p.) or with vehicle. Afterwards, levels of different markers (TH, DAT, GFAP, IBA-1, caspase) were evaluated in the medial pre-frontal cortex (mPFC), nucleus accumbens (NAc), caudate-putamen (CPu) and ventral tegmental area (VTA) as signs of JWH-018-induced neurodegeneration and neuroinflammation. Moreover, studies on anxiety-like (Elevated Plus Maze, EPM), and/or repetitive-like behaviors (Marble Burying, MB), and attentional processes (Prepulse Inhibition, PPI) were performed.

Results showed that JWH-018 treatment increases IBA-1 immunoreactivity in the NAc core and reactive astogliosis (GFAP) in the mPFC and in the NAc shell.

Behavioral data showed that JWH-018 treatment increases anxiety-like states and repetitive-like behaviors as revealed by a decreased time spent in the open arms of the EPM and by the higher number of marbles buried in the MB test, and impairs the PPI of the acoustic startle reflex.

These results allow to speculate that the activation of microglia and astrocytes, that are an index of neuroinflammation, might be related to the behavioral central effects observed after the treatment with JWH-018.

P13.17 IMPACT EVALUATION SURVEY: AFTER COMPLETION OF EURO-MEDITERRANEAN MASTER IN NEUROSCIENCE AND BIOTECHNOLOGY ONLINE

Zanchetta MS[1], **Landry M**[2] **and Mésenge C**[3]
[1]*Ryerson University, Toronto, Canada*
[2]*ISIS Euro-Mediterranean Master in Neuroscience and Biotechnology, Université de Bordeaux, France*
[3]*Université Numérique Francophone Mondiale, France*

The social impact of the Euro-Mediterranean Master in Neuroscience and Biotechnology graduates' roles in their scientific environment of several Euro-Mediterranean countries remain unidentified. The Master is multidisciplinary integrating 11 partner universities located in France, Italy, Spain, Egypt, Lebanon and Morocco.

Principles of inter-professional education, such as cooperation, teamwork, flexibility, sociopolitical influence, and expertise among other actions as well as the higher parameters of learning goals for innovation and creation will compose the conceptual framework. Data collection will use Opinio, a survey online platform hosted by Ryerson. The survey has the following objectives: (a) Identify the extent of social impacts related to the use of refined professional skills towards the development of scientific expertise and practice by the Master's graduates; (b) Analyse the contribution of the Master's graduates to the professional contexts of neuroscience and biotechnology in Euro-Mediterranean countries; and (c) Enlighten Master's graduates' practice and roles by revealing their trends and knowledge gaps so as to inspire the creation of future inter-professional training programs in the fields of neuroscience and biotechnology that are able to respond to the timely scientific and societal trends in the target countries.

They are expected to portray graduates' views of the extend of refinement of professional objectives and achievements, integration into the labor market, participation in a research community, change on ways of thinking, of understanding their practice setting and the nature of their professional service to the local and scientific communities. They will indicate which new attitudes, knowledge, awareness, skills, motivation and intentions have been influenced by the Master.

Key aspects regard to international partnerships in evaluative research within global perspectives of changes in professional practice in countries with unequal work conditions.

Participants' accounts will equally inform curriculum redevelopment as well as new areas for consolidating and expansion.

P13.18 "OXIDATIVE STRESS-INFLAMMATION-APOPTOSIS" NETWORK IN THE PATHOGENESIS OF ISCHEMIC STROKE

Tsakanova G[1,2], **Arakelova E**[1], **Ayvazyan V**[1], **Ayvazyan A**[2], **Boyajyan A**[1] **and Arakelyan A**[1]
[1]*Institute of Molecular Biology, NAS RA, Yerevan, Armenia*
[2]*CANDLE Synchrotron Research Institute, Yerevan, Armenia*

Oxidative stress (OS), postischemic inflammatory response and apoptosis are the key pathogenic factors leading to uncontrolled cell damage and death, which badly influences ischemic stroke (IS) progression and outcome. The molecular mechanisms involved in the development of these processes are not clear yet, which is limiting the identification of therapeutic targets for IS.

The aim of this study was to reveal the molecular mechanisms responsible for the development of OS, inflammatory reactions and apoptosis in human IS on the systemic level and to identify the molecular components involved in these processes. The levels of lipid hydroperoxides, lipofuscin, 3-nitrotyrosine, 8-isoprostaglandine-F2α, matrix metalloproteinases-9, and 8-hydroxi-2′-desoxiguanosine, mannan-binding lectin (MBL), ficolins H and L as well as the activities of mannan-associated serine proteases 1 and 2 (MASP-1, MASP-2), apoptotic marker annexin-A5, the total capacity of antioxidants (TAC), ferroxidase activity of ceruloplasmin (FAC) and the activities of superoxide dismutase and catalase, as well as the possible association of IS with the ANXA5 gene *rs11575945* (-1C/T) were assessed by using blood serum, hemolyzed erythrocytes and DNA samples of IS-affected and healthy subjects (HS).

The results obtained demonstrated an increase in the TAC and FAC, as well as in levels of lipid hydroperoxides, MBL, MASP-1, MASP-2 and annexin-A5 in response to OS at day 1 of IS-onset. In addition, it was shown that the *rs11575945* (-1C/T) polymorphism of the annexin-A5 gene is associated with IS.

Based on the data obtained, we concluded that ischemic stroke is characterized by the alterations in the "oxidative stress-inflammation-apoptosis" network, and

that lipid hydroperoxides, TAC, ceruloplasmin, MBL, MASP-1, MASP-2 and annexin A5 may be considered as targets for targeted therapeutic correction of ischemia induced inflammatory reactions and destructive processes in IS.

P13.19
PHENOTYPIC OVERLAP BETWEEN DISRUPTION OF SNRNP BIOGENESIS AND SMN-GEMINS COMPLEX PERTURBATION: IMPLICATIONS FOR MOTOR NEURON DISEASE

Borg R[1,2,3], Lanfranco M[1,2,3], Cacciottolo R[1,2], Camilleri M[1,2], Bordonne R[3], Cauchi RJ[1,2]

[1]*Department of Physiology and Biochemistry, Faculty of Medicine and Surgery, University of Malta, Msida, Malta*

[2]*Centre for Molecular Medicine and Biobanking, Biomedical Sciences Building, University of Malta, Msida, Malta*

[3]*Institut de Montpellier, CNRS-UMR5535, France*

The neuromuscular disorder, spinal muscular atrophy (SMA), is the result of insufficient levels of the ubiquitously-expressed survival motor neuron (SMN) protein. SMN associates with Gemins 2-8 and Unrip to from the large macromolecular complex. The SMN-Gemins complex is key for chaperoning the coupling of a heptameric ring of Sm proteins with small nuclear RNAs thereby generating small nuclear ribonucleoproteins (snRNPs), the core constituents of the spliceosome. The early snRNP assembly phase involves post-translational modification and the formation of two distinct Sm protein sub-complexes each sharing pICln, which prevents premature RNA interactions. In the late snRNP assembly phase, the 7-methylguanosine cap of assembled snRNPs is hypermethylated by trimethylguanosine synthase 1 (Tgs1). The modified cap together with the Sm ring act as a nuclear-localisation signal. In view of non-canonical functions linked to the SMN-Gemins complex including a role in the axonal trafficking of mRNAs, it is still presently unclear how defects in snRNP assembly can be reconciled with the selective neuromuscular degeneration that is typical in SMA. Attempting at addressing whether the involvement of the SMN-Gemins complex in snRNP biogenesis is imperative for a functional neuromuscular system *in vivo*, we examined phenotypes resulting from the disruption of pICln or Tgs1, two cardinal players in the snRNP biogenesis pathway, which have never been directly linked to axonal metabolism. Intriguingly, we uncover that deviations from normal protein levels in muscle tissue including overexpression of full-length pICln or enhanced Tgs1 knockdown results in adult flies with severe motor system defects, in contrast to controls but similar to flies with either muscle-restricted Gemin3 knockdown or SMN loss-of-function. We also demonstrate a genetic and physical interaction between Gemin3 and pICln or Tgs1 with such findings confirming that members of the SMN-Gemins complex work closely with snRNP assembly factors *in vivo*. Interestingly, we find that overexpression of either pICln or Tgs1 was by itself sufficient to cause motor dysfunction in *Drosophila*. Toxicity is conserved in the yeast *S. pombe* where overexpression induces a surplus of Sm proteins in the cytoplasm, indicating that a block in snRNP biogenesis is partly responsible for this phenotype. We propose that snRNP biogenesis is the pathway connecting the SMN-Gemins complex to a functional neuromuscular system, and its disturbance most likely leads to the motor dysfunction that is typical in SMA. Considering the intersection of the SMN-Gemins complex with amyotrophic lateral sclerosis (ALS) our findings have broader implications on our understanding of the mechanisms underpinning motor neuron disease.

P13.20
AMPHETAMINE AND THE 'BATH SALT' MDPV ENHANCE GENERALIZATION OF MEMORY FOR EMOTIONAL EXPERIENCES IN RATS

Colucci P[1], Mancini GF[1], Santori A[1], Roozendaal B[2] and Campolongo P[1]

[1]*Department of Physiology and Pharmacology, Sapienza University of Rome. Rome. Italy*

[2]*Department of Cognitive Neuroscience, Radboud University Medical Centre, Nijmegen, Netherlands*

The mechanisms by which drugs of abuse affect the accuracy of memory processes are not well understood. Here we tested the effects of the psychostimulants Amphetamine and the "bath salt" MDPV in an inhibitory avoidance discrimination task. Male SD rats (350–370 g) were trained and tested in different apparatuses. During training rats were placed into the light compartment of a first inhibitory avoidance (Non-Shock box) and they were allowed to cross to the dark compartment. Then, after a 1-min delay, they were placed into the light compartment of a second, contextually distinct, inhibitory avoidance apparatus (Shock box), and they received footshock upon entering the dark compartment. Amphetamine (1–3 mg/kg), MDPV (0.5–1.0 mg/kg) or saline were administered (i.p.) immediately after training. On the 48-h retention test, rats were tested, in a randomized order, in the Shock and Non-Shock boxes as well as in a Novel box.

Controls had similar retention latencies in the Shock and Non-Shock boxes, indicating lack of discrimination. However, latencies in the Shock and Non-Shock boxes were longer than those in the Novel box, indicating that rats recognized the two training contexts. Amphetam-

ine (3 mg/kg) increased retention latencies in the Shock box indicating an increase in memory strength. However, retention latencies in both safe environments were also increased, indicating an increased generalization. MDPV did not enhance memory, but it (1 mg/kg) increased retention latencies in the Novel box, inducing generalization.

Amphetamine and MDPV had differential effect on memory strength, but both drugs increased generalization of memory for emotional training. It is tentative to hypothesize that the different effects on memory strength versus generalization could be due to differences in the modulation of the monoaminergic neurotransmissions, in the recruitment of different brain areas or in the interaction with the stress response systems.

P13.21
THE EFFECT OF DISULFIRAM ON MORPHINE SELF-ADMINISTRATION AND REINSTATEMENT OF SEEKING BEHAVIOR IN RATS

Frankowska M[1], Kleczkowska P[2], Suder A[1], Kluska K[1], Bujalska-Zadrożny M[2] and Filip M[1]

[1]*Institute of Pharmacology, Polish Academy of Sciences, Laboratory of Drug Addiction Pharmacology, 12 Smętna, 31-343 Krakow, Poland*

[2]*Medical University of Warsaw, Centre for Preclinical Research and Technology, Department of Pharmacodynamics, 1B Banacha, 02-097 Warsaw, Poland*

Disulfiram (DSF), the anti-alcoholism drug, is also effective in the treatment of cocaine addiction and in cocaine and alcohol co-abuse. Up to now, there are no reports indicating the effects of DSF on the behavioral actions of opioids. In our preliminary studies we have shown that DSF alleviates the symptoms of withdrawal after methadone or morphine treatment and reduces tolerance to the analgesic effects of these opioids in acute pain model.

In the present study we investigated the effect of DSF on morphine self-administration and reinstatement of seeking behavior in rats.

Male Wistar rats were trained to self-administer morphine (0.5 mg/kg/inf.) and each drug infusion was associated with contextual cue (light+tone). Another group of rats underwent morphine self-administration and extinction training. After 14 days of extinction, reinstatement of responding was induced by morphine (2.5–5.0 mg/kg, ip) or drug associated cue. For statistical analyses a multi-way ANOVA with repeated measures and post-hoc Newman-Keuls' test were used.

During maintenance of morphine self-administration we found that acute treatment with DSF 12.5–50.0 mg/kg (ip) dose-dependently attenuated behavioral responding of rats, with significant reduction in the number of active lever presses and drug infusions. Moreover, at 6.25–25.00 mg/kg DSF decreased morphine seeking-behavior triggered by morphine priming or cues in rats previously administered morphine. Daily pretreatment with DSF (50 mg/kg) during extinction non-significantly reduced behavioural responses at first three drug-free days. Furthermore, repeated treatment with DSF during extinction did not affect the following reinstatement of drug-seeking behavior in rats.

Our findings show that the DSF may hold promise as potential pharmacotherapies for morphine addiction as applied in the form of acute prevention for morphine relapse.

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P13.22
AGGRESSIVE BEHAVIOR AND SOMATIC PROBLEMS IN MOROCCAN STUDENTS REPORTING ABUSE AND ALCOHOLISM IN THEIR HOME

Zouini B[1], Sfendla A[1], Senhaji M[1] and Kerekes N[2]

[1]*Department of Biology, Faculty of Sciences, Abdelmalek Essaadi University, Tetouan, Morocco*

[2]*Department of Health Sciences, University West, Trollhättan, Sweden*

Increased aggressive behaviour in youngsters has been coupled to the development of several negative outcomes in adulthood, such us substance use, personality disorder and criminality. Background factors explaining their deviant behaviour include both familiar and environmental factors. The aim of the present study was to compare the prevalence of atypical aggression and defined somatic problems in those children who have reported alcohol use or psychological/physical abuse at their home with these factors in.

Self-reported information from 280 (145 boys and 135 girls, ages between 15 and 18) Moroccan students were assessed during the on-going international project: "Mental and Somatic Health without borders (MeSHe)". The MeSHe survey includes, between other mental health inventories, measure of aggressive trait and gathers information about somatic health and social environmental factors.

Almost 70% of the students who reported alcohol-use problem or abuse at home also reposted headaches, and almost every fourth of them reported migraine. Of those who experienced abuse at home significantly more also indicated complains for diarrhoea or constipation, and significantly more had allergies. They had also signific-

antly increased risk of having an atypically high level of aggression themselves (54% increase when family they had alcohol use in their family was reported and 77% increase when psychological/physical abuse accord in their family).

The presence of alcoholic parents or physical/psychological abuse in adolescence, increase the risk of atypical aggression and the risk for coexisting somatic complains.

P13.23
PLA2G4E PRODUCES A C LASS OF ENDOCANNABINOID PRECURSORS IN MOUSE BRAIN

Margiani G[1], Parsons WH[2,3], Potter Z[2], De Luca MA[1] and Cravatt BF[2,3]

[1]*Department of Biomedical Sciences, University of Cagliari, Italy*

[2]*Department of Chemical Physiology, The Scripps Research Institute, La Jolla, California, USA*

[3]*The Skaggs Institute for Chemical Biology, The Scripps Research Institute, La Jolla, California, USA*

PLA2G4E is a serine hydrolase recently shown to produce N-acylphosphatidylethanolamines (NAPEs) through a calcium-dependent acyl transfer reaction when overexpressed in mammalian cells. NAPEs represent precursors of N-acyl ethanolamines (NAE), a class of bioactive lipids that includes the endocannabinoid anandamide (AEA). Though much is known about the degradation of NAEs, their biosynthesis is less well understood. In order to investigate a potential role for *PLA2G4E* in the biosynthesis of endocannabinoids, we generated and characterized mice with targeted disruption of the *Pla2g4e* gene.

Prior studies indicate that PLA2G4E is primarily expressed in mouse brain, heart, skeletal muscle, and testis. For our experiments, these tissues were collected from PLA2G4E$^{+/+}$ and PLA2G4E$^{-/-}$ mice and processed to obtain their membrane proteomes. These proteomes were first studied by activity-based protein profiling (ABPP) using serine hydrolase-selective fluorophosphonate probes and were then tested for their ability to catalyze the synthesis of *N*-C16:0 NAPE in the presence and absence of CaCl2 using an LC/MS assay.

The results from our SDS-PAGE and LC/MS experiments support successful knockout of PLA2G4E and confirm no other significant variations in the expression levels of serine hydrolases in these tissues. Further, we found that calcium-dependent NAPE production in PLA2G4E$^{-/-}$ brain proteomes was reduced by $> 90\%$ compared to PLA2G4E$^{+/+}$ proteomes. These data support the assignment of PLA2G4E as the primary enzyme responsible for the calcium-dependent generation of NAPEs in brain.

The endocannabinoid system is involved in many functions, including brain development and stress. Understanding the physiological role of PLA2G4E in the biosynthesis of NAEs will provide important insight into the production of endocannabinoids and potentially inform our understanding and treatment of abnormal endocannabinoid signaling in disorders like neurodegeneration and addiction.

P13.24
NANOSCALE IMAGING OF VENTRAL TEGMENTAL AREA DOPAMINE CELL INPUTS FOLLOWING PRENATAL Δ^9-TETRAHYDROCANNABINOL EXPOSURE

Sagheddu C[1], Miczán V[2,3], Katona I[2], Melis M[1]

[1]*Department of Biomedical Sciences, Division of Neuroscience and Clinical Pharmacology, University of Cagliari, Monserrato, Italy*

[2]*Institute of Experimental Medicine, Hungarian Academy of Sciences, Budapest, Hungary*

[3]*Faculty of Information Technology and Bionics, Pázmány Péter Catholic University, Budapest, Hungary*

Marijuana consumption during pregnancy has been associated with impairments in fetal brain, and with cognitive and behavioral disruptions long after birth. In the brain, the psychoactive compound of marijuana, Δ^9-tetrahydrocannabinol (THC), activates cannabinoid receptor 1 (CB1), which is expressed on presynaptic terminals and modulates neurotransmitter release. As a part of the endocannabinoid system, CB1 play a pivotal role in neuronal differentiation and synaptogenesis. However, preclinical studies investigating synaptic molecular changes in reward-related areas following marijuana exposure are still elusive.

In this study, rat dams were administered THC (2 mg/kg), or its vehicle, once per day from gestational day 5 to 20. Correlated confocal/STochastic Optical Reconstruction Microscopy (STORM) was performed to image with nanoscale precision synaptic changes of offspring ventral tegmental area (VTA) during peri-adolescence (PND 21-29). Particularly, immunostaining for tyrosine hydroxylase (TH) and for vesicular glutamate 1 (VGluT1) or GABA transporter (VIAAT) allowed visualization of dopamine cells and excitatory or inhibitory afferents, respectively. Concurrent immunostaining for CB1 or bassoon allowed STORM-based molecular quantification within boutons.

In the offspring of THC-exposed dams, we found a reduced number of TH positive cells and reduced bouton area in the vGluT1, but not VIAAT, afferents impinging onto these neurons. CB1 content of both vGluT1- and

VIAAT-positive axon terminals was not changed. Consistently, CB1 density in vGluT1 containing boutons was higher following prenatal THC exposure. Number and clustering of bassoon STORM localization points were the same in THC and control groups, suggesting that the active zone structure remained intact.

Our results show that exposure to marijuana during early development perturbs reward-related areas in the brain, which might result into increased vulnerability toward psychiatric disorders, later in life.

P13.25
NEUROANATOMICAL CHANGES ASSOCIATED WITH A MINDFULNESS-BASED INTERVENTION IN INDIVIDUALS WITH ATTENTION DEFICIT HYPERACTIVITY DISORDER (ADHD): DESIGN AND RATIONALE FOR A CONTROLLED TRIAL

Mendrek A[1], Poissant H[2], Whittingstall K[3] and Descoteaux M[4]

[1]*Dept. Psychology, Bishop's University, Montréal, Canada*

[2]*Dept. Education & Pedagogy, Université de Quebec a Montréal, Montréal, Canada*

[3]*Dept. Diagnostic Radiology, Université de Sherbooke, Montréal, Canada*

[4]*Dept. Computer Science, Université de Sherbooke, Montréal, Canada*

The prevalence of the attention deficit hyperactivity disorder (ADHD) has been on the rise over the past decades. The reasons for this increase are complex and the expression of the disorder is heterogeneous, ranging from mild to severe disturbances in attention, cognition, motivation and emotion regulation. The most common treatment consists of administration of stimulants, such as methylphenidate and amphetamine. However, researchers and clinicians started exploring alternative modes of treating ADHD including mindfulness-based interventions (MBIs). Our recent meta-analysis shows that these MBIs may be moderately effective in reducing symptoms of ADHD. However, we do not know anything about the underlying neural mechanisms of this effect. Thus, the purpose of the present study is to examine neurocognitive effects of an intensive 8-week MBI on young adults with ADHD in comparison to matched healthy controls. Participants will undergo cognitive assessment, as well as magnetic resonance imaging (MRI) to evaluate structural (using diffusion tensor imaging – DTI) and functional (using resting-state fMRI) connectivity, before and after the intervention. We expect to find improvements on measures of attention, executive function and emotion regulation, in both groups, though the gains should be greater in individuals with ADHD than in controls. At the neural level, we expect to find strengthening of the central executive network (CEN) and reorganization of the default-mode network (DMN) and salience network (SN), which will be apparent especially in the ADHD group. The results should reveal the neural mechanisms underlying effectiveness of MBI for ADHD, thus reinforcing the case for using it in addition, or instead of, medication in this disorder.

P13.26
PRENATAL STIMULATION OF THE ENDOCANNABINOID SYSTEM AND THE CONSEQUENCES ON THE MOTHER-INFANT DYAD: A PRECLINICAL REMARK

Lavanco G[1], Brancato A[1], Cavallaro A[1], Leonardi D[1], Plescia F[1], Melis M[2] and Cannizzaro C[1]

[1]*Department of Sciences for Health Promotion and Mother and Child Care "G. D'Alessandro" University of Palermo, Italy*

[2]*Division of Neuroscience and Clinical Pharmacology, Department of Biomedical Sciences, University of Cagliari, Italy*

The dam-infant interaction is a fundamental relationship, functionally necessary to the infant's wellness and to the development of his own behavioural and physiological repertoire. Drugs exposure during pregnancy induces detrimental consequences, that are not limited to the direct in utero effects of the drug on fetuses, but also extend to maternal care. The present study investigates the effects induced by prenatal stimulation of endocannabinoid system on maternal behaviour along postnatal period. Female rats received a stimulation of the endocannabinoid system from gestational day 5^{th} to 20^{th}. Maternal care was assessed by recording dams' undisturbed spontaneous home-cage behaviour in the presence of their offspring. Our results show that prenatal stimulation of endocannabinoid system induced a deescalation in maternal behaviour with respect to vehicle over the postnatal period ($p < 0.001$). Furthermore, the prenatally treated dams showed a significant increase in single non-maternal behavioural categories, such as self-grooming, dam self-care, rearing and general arousal when compared to controls ($p < 0.01$; $p < 0.001$). The outcomes of this study show that prenatal exposure to cannabinoids reduces the dams-offspring attachment and underlie the importance of modelling human drug habit and its consequences on the mother-infant dyad, in order to prevent detrimental effects on offspring development and maturation.

P13.27
EPIGENETIC REGULATION OF ADENOSINE A_{2A} AND DOPAMINE D2 RECEPTOR GENE TRANSCRIPTION ON COMPULSIVE FOOD CONSUMPTION

Cifani C[1], Micioni Di Bonaventura MV[1], Pucci M[2], Giusepponi ME[1], Lambertucci C[3], Romano A[4], Volpini R[3], Maccarrone M[5] and D'Addario C[2]

[1] *University of Camerino, School of Pharmacy Pharmacology Unit, Camerino (MC), Italy*
[2] *University of Teramo, Faculty of Bioscience and Technology for Food Agriculture and Environment, Teramo, Italy*
[3] *University of Camerino, School of Pharmacy Medicinal Chemistry Unit, Camerino (MC), Italy*
[4] *Department of Physiology and Pharmacology, Sapienza University of Rome, Rome, Italy*
[5] *University of Rome, Campus Bio-Medico, Rome, Italy*

Satisfactory treatments for eating disorders, such as binge eating disorder and bulimia nervosa, are not available at present. Using a well-characterized animal model of binge eating, we investigated the epigenetic regulation of the adenosine A_{2A} receptor (A_{2A}AR) and dopamine D2 receptor (D2R) gene.

The animal model included four groups (rats fed normally, and then stressed or not, rats exposed to cycles of restriction/refeeding, and then stressed or not).

Gene expression analysis carried out on the amygdala complex of restricted and stressed rats revealed a significant increase of A2AAR and D2R mRNA when compared to non-stressed and non-restricted rats. Administration of the A_{2A}AR agonist (VT 7) induced in restricted and stressed rats a significant increase of A_{2A}AR and D2R mRNA levels when compared to vehicle group, whereas a significant decrease in rats pre-treated with the A_{2A}AR antagonist (ANR 94) was observed.

Pyrosequencing analysis revealed a significant reduction of the % of DNA methylation at A_{2A}AR promoter region in restricted and stressed rats compared to the non-stressed and non-restricted animals. We did not find any difference in D2R DNA methylation among different groups. Significant changes in the DNA methylation status of A_{2A}AR promoter were found in restricted and stressed rats after administration of VT 7 or ANR 94. We observed a decrease of DNA methylation in VT 7 treated rats and a hypermethylation in ANR 94 rats with respect to the vehicle group. The increase in A_{2A}AR mRNA observed in restricted and stressed rats could be due to a compensatory mechanism to counteract the effect of binge eating, suggesting that the A_{2A}AR activation, inducing receptor gene up-regulation, could be relevant to reduce food consumption. We here demonstrated for the first time the epigenetic regulation of A_{2A}AR in an animal model of binge eating.

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P14.1
AN INVESTIGATION OF THE ROLE OF CHANGES IN THE VAGUS NERVE IN OBESITY INDUCED BY A HIGH-FAT DIET: A STEREOLOGICAL AND IMMUNOHISTOCHEMICAL STUDY

Arslan G, Alkan I and Altunkaynak BZ
Department of Histology and Embryology, Medical School, Ondokuz Mayıs University, Samsun, Turkey

Developing technology and the increasing pace of life have reduced our need to move, while adding high-calorie foods to our diet. Deleterious changes in eating habits cause the calorie balance in the body to deteriorate. Increased calorie intake and a decrease

in calorie expenditure causes this balance to deteriorate toward excess calories and results in these being stored as fat reserves in the body. This then results in obesity. Obesity accompanying cardiovascular diseases, diabetes, psychological disorders, biliary and many other diseases prepares the ground for potentially fatal risks.

This study was intended to investigate how vagus nerve operations alter body weight, as well as the hunger and satiety centres, and to examine the relationship between the hypothalamus and the vagus nerve. Thirty-two obese *Wistar albino* rats were randomly divided into four groups - control, sham, inhibition and stimulation. No procedure was performed in the control group. In the sham group, the left vagus nerve was opened and closed with a 1 cm midline incision 2 cm below the neck region. The neck area of the subjects from the stimulation group was opened using the same surgical technique, and a special stimulator was attached to the left vagus nerve. Postoperative investigation began on the second day and lasted for 4 weeks, with vagal stimulation being applied for 5 minutes a day at a frequency of 30 Hz/500 ms/30 seconds. In the inhibition group, a similar incision was made, and the nerve was tightened for 30 seconds using a special clamp capable of applying 58 Newtons of pressure for damage output. After surgery, subjects were left to heal for 4 weeks. At the end of the 13th week of the experiment, all subjects were perfused, and the number of myelinated axon fibers, the average myelin thickness in vagus nerve samples, and total numbers of neuron nuclei within the hypothalamus were estimated using stereological techniques. Neurons in the hypothalamic nuclei were evaluated immunohistochemically with anti-neuropeptide Y and anti-POMC.

Arcuate, ventromedial and dorsomedial nuclei were analysed in the hypothalamic field. The mean number of neurons in the arcuate and ventromedial nucleus areas increased in the stimulation group. The mean number of neurons in the dorsomedial nucleus area decreased. The mean number of neurons decreased in all nucleus areas in the inhibition group. The number of myelinated axons increased in the inhibition group, while myelin sheath thickness and myelinated axon area decreased considerably at analyses of vagus specimens. No significant differences were observed in terms of other parameters between the other groups. Immunohistochemical analysis revealed the most active cells in the stimulation and the inhibition groups, while NPY active cells were observed in the control and sham groups. The greatest weight loss was determined in the inhibition group.

In conclusion, the neuronal increase observed in the arcuate and ventromedial nucleus areas is probably due to the secretions of these two nuclei, which regulate energy metabolism, and suggests that the stimulation mechanism in obesity occurs mainly through ventromedial nucleus secretions.

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P14.2
EFFECTS OF TOPIRAMATE ON THE VENTROMEDIAL AND DORSOMEDIAL NUCLEI AND HYPOTHALAMIC LEVELS OF OBESITY-ASSOCIATED PROTEIN IN OBESE RATS

Alkan I, Altunkaynak BZ and Kocaman A
Department of Histology and Embryology, Medical School, Ondokuz Mayıs University, Samsun, Turkey

Obesity has become one of the most common health problems and the cause of many diseases. Investigation of the mechanisms involved in obesity has revealed that food intake and appetite are regulated by the activities of the hypothalamic nuclei, including the brain's hunger and satiety areas. This study investigated the effects of topiramate on weight loss in the hypothalamic nuclei. Fat-mass protein (FTO) levels were also investigated in these nuclei. Twenty four female *Wistar albino* rats were randomly divided into four groups of six subjects each - obese, obese-topiramate, control and control-topiramate. The obese groups received a 40% high-fat diet, while the control groups were given a standard diet throughout the study. Topiramate was administered at 0.02 mg/kg once daily for six weeks in the topiramate treatment group. At the end of six weeks, the animals were sacrificed with intracardiac perfusion, and their brain tissues were removed. The extracted brain tissues were subjected to routine histological procedures, and serial sections 20 microns in thickness were taken from the brain tissues of five animals for stereological analysis. Sections seven microns in thickness were taken from the brain tissues for immunohistochemical analysis from one rat randomly selected from each group.

Hypothalamic nuclei in the sections taken for stereological analysis were analysed using optical fraction methods. Immunohistochemical staining was performed using anti-FTO antibody for FTO concentrations in the nuclei. The mean number of neurons in the dorsomedial nucleus was significantly lower in the obese topiramate group than in the obese control group. No significant difference was observed between the control and control topiramate groups. The mean number of neurons in the ventromedial nuclei was significantly lower in the control group than in the other groups. The mean number of neurons increased in the obese control and control topiramate groups compared to the control group. No difference was observed between the obese control and obese topiramate groups. The largest number of FTO positive cells was observed in the obese control group and the lowest number in the topiramate control group. The results show that obesity and anti-epileptic drugs used for treatment produce different effects on the two nuclei.

We attribute this variation to the role of dorsomedial and ventromedial nuclei in the regulation of energy metabolism and body heat and the various roles played by obesity. In addition, the results are significant in terms of topiramate's weight-loss effect being based on the reduction of body heat or suppression of certain fasting proteins. FTO density study confirmed that FTO expression was increased by obesity, supporting the hypothesis that the effect of topiramate may be related to fasting proteins.

The relationship between obesity and topiramate now needs to be clarified through detailed pathway analysis of the nuclei and FTO gene expression.

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P14.3
ACTIVATION OF HYPOTHALAMIC 5-HT$_1$ AND 5-HT$_2$ RECEPTORS DIFFERENTLY REGULATES ENDOCRINE RESPONSES AND LIVER CYTOCHROME P450 EXPRESSION AND ACTIVITY

Daniel WA, Haduch A, Bromek E, Rysz M and Wójcikowski J
Polish Academy of Sciences, Institute of Pharmacology, Kraków, Poland

Our earlier work showed that brain serotonergic system affected the expression of cytochrome P450 (CYP). Serotonergic innervation of the hypothalamic paraventricular nuclei (PVNs) had a positive effect, while

that of the arcuate nuclei (ARCs) had a negative impact on growth hormone (GH) secretion and GH-dependent CYP expression. The aim of the present study was to identify 5-HT receptor types in the PVN and ARC, engaged in the regulation of liver CYP expression and activity.

The experiment was carried out on male Wistar rats. Intracerebral injections of 5-HT_1 receptor agonists (5-CT – 5-HT_1 agonist; 8-OH-DPAT – 5-HT_{1A} agonist; sumatriptan – 5-$HT_{1B/D}$ agonist) or the 5-$HT_{2A/2C}$ receptor agonist DOI into the PVN or ARC were performed for five days. Liver CYP expression (mRNA, protein) and activity (testosterone hydroxylation), as well as pituitary and serum hormones were measured.

When injected into the PVN, 5-CT significantly decreased the expression and activity of the isoenzymes CYP2C11 and CYP3A, being accompanied with an increase in pituitary somatostatin and a decrease in serum GH concentration. Similar effects were observed after 8-OH-DPAT. Sumatriptan or DOI had no effect on liver CYP. In contrast, DOI, but not 5-CT, injections into the ARC caused a significant increase in the expression and activity of CYP2C11 and CYP3A.

The results indicate that – *via* stimulation of somatostatin release and a consequent decrease in GH secretion in the pituitary – 5-HT_{1A} receptors of the PVN negatively regulate CYP2C11 and CYP3A. On the other hand – via stimulation of the secretion of GH releasing hormone and the resulting increase in GH release – 5-HT_2 receptors of the ARC positively regulate CYP2C11/3A (60% of total CYP).

The obtained results show involvement of the 5-HT_{1A} receptors of the PVN in the negative regulation, and the role of the 5-$HT_{2A/C}$ receptors of the ARC in the positive regulation of liver cytochrome P450 expression and activity via neuroendocrine mechanisms.

P14.4
THE VISIBLE BURROW SYSTEM: A NEW BEHAVIOURAL PARADIGM TO ASSESS SOCIAL WITHDRAWAL IN GROUP HOUSED RODENTS

Bove M[1,2], Ike K[1], Eldering A[1], Buwalda B[1], De Boer S[1], Kas MJ[1]

[1] *Groningen Institute for Evolutionary Life Science, University of Groningen, The Netherlands*

[2] *Department of Physiology and Pharmacology "V. Erspamer", "Sapienza" University of Rome, Italy*

Social withdrawal is an early symptom of a wide variety of neuropsychiatric diseases, such as schizophrenia, autism spectrum disorders, depressive disorders and Alzheimer's disease. The paucity of objective measures to longitudinally assess social withdrawal characteristics has been an important limitation to study this behaviour, both in human and rodents. Although a small number of studies have identified ways to study social group behaviour in rodents in a longitudinal manner, it is currently unknown whether outcome measures from these paradigms will be relevant for social withdrawal behaviour observed in neuropsychiatric patients.

The aim of the present work was to study social withdrawal in rodents using a new behavioural paradigm, the Visible Burrow System (VBS). The VBS mimics a natural environment, with male and female rodents housed together in an enclosure where an open arena is connected to a continuously dark burrow system that includes 4 boxes connected by corridors. In this study, mixed-sex colonies of C57BL/6J and of BTBR mice have been investigated.

Results showed marked differences between the two strains, in terms of social interactions as well as non-social behaviours, pointing out the advantage of the use of VBS to study social group behaviour dynamics that naturally occur in a mixed-sex colony.

In conclusion, this social housing model appears to be a powerful tool to longitudinally study social withdrawal aspects that may be relevant in neuropsychiatric disorders.

P14.5
TIME DEPENDENT EFFECTS OF RESTRAINT AND CROWDING STRESS ON THE EXPRESSION OF GLUTAMATE RECEPTORS IN THE RAT PREFRONTAL CORTEX

Zelek-Molik A[1], Gądek-Michalska A[2], Bobula B[2], Chorązka K[1], Hess G[2] and Nalepa I[1]

[1] *Department of Brain Biochemistry*

[2] *Department of Physiology, Institute of Pharmacology, Polish Academy of Sciences, Krakow, Poland*

Stress, depending on its intensity, nature and time of exposure, can evoke maladaptive changes and lead to stress related disorders. Disturbance in glutamatergic transmission in prefrontal cortex (PFC) is considered to underlie this pathology. In presented study we measured GluA1, GluN2B and mGluR1a/5 expression in PFC of rats that underwent different time of exposure to stress of crowding (CS) or restraint (RS). Furthermore, we assessed how sub-chronic CS affects basal synaptic transmission and induction of LTP.

Three main experimental groups of male Wistar rats were studied: control, CS and RS. Stress procedures were performed for 3, 7 or 14 days. Protein expression was assessed by Western Blot. Electrophysiological recordings of field potentials and LTP were done in layer II/III after stimulation of layer V.

Electrophysiological results revealed increased relation between stimulus intensity and field potential amplitude together with attenuated LTP in CS-3d rats. Ana-

lysis of protein expression has shown in CS-3d increased level of GluA1, GluN2B and mGlu1a/5 (respectively by 50, 112 and 56% vs. control). Similar increase ($\sim$ 50% vs. control) was also observed in RS-7d group. In contrast, chronic (14d) CS and RS attenuated the level of GluA1, GluN2B and mGluR1a/5 ($\sim$ 30% vs control).

Our data demonstrate that stress triggers dynamic and time-dependent changes in the level of glutamate receptor proteins. Because GluA1 and GluN2B are subunits that modulate membrane stability of AMPA and NMDA receptors, their augmented expression can explain increased basal activity of PFC neurons after subchronic stress. LTP reduction in this group suggests that observed basal over-activation is reversely correlated with synaptic strengthening in PFC. Prolonged stress-evoked decrease of glutamate receptor proteins may contribute to PFC hypofunction observed in stress related disorders.

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P14.6
CHRONIC STRESS EXPOSURE INDUCED THE DEVELOPMENT OF COGNITIVE DEFICITS: INVOLVEMENT OF GENOMIC VS NON-GENOMIC EFFECT MEDIATED BY GLUCOCORTICOID RECEPTORS

Brivio P[1], Papp M[2], Racagni G[1], Riva MA[1], Calabrese F[1]

[1] *Department of Pharmacological and Biomolecular Sciences, University of Milan, Milano, Italy*

[2] *Institute of Pharmacology, Polish Academy of Sciences, Krakow, Poland*

Psychiatric diseases are characterized by an altered function of the HPA axis. Moreover, this system is also involved in learning and memory processes and its deregulation may lead to the development of cognitive deficits. Corticosteroids, in the brain, activate the glucocorticoid receptors (GR) and the mineralocorticoid receptors (MR) both at nuclear and membrane level.

Hence, we investigated the effect of the exposure to the chronic mild stress (CMS) on the cognitive performance and, at molecular levels, on the genomic vs non genomic activity of GR and MR in the dorsal hippocampus. Wistar rats were exposed to CMS (7 weeks), tested at weekly intervals with sucrose consumption test to assess anhedonia. At the end of the CMS, the animals were exposed to novel object recognition (NOR) test. mRNA levels analysis of GR responsive genes were carried out by Real-Time PCR, whereas Western blot was used to conduct protein assay.

Rats exposed to CMS develop an anhedonic phenotype and an impairment in the cognitive performance assessed in the NOR test. At molecular level, CMS per sè increased GR protein levels in the membrane compartment, effect paralleled by an up-regulation of SINAPSYN Ia/b phosphorylation in Ser603. Differently, NOR test exposure induced a significant increase of GR protein levels in the nucleus of no stress rats and this increase mirrors an up-regulation of the transcriptional activity of GR as demonstrated by the effect observed on Gadd45b and Sgk-1 mRNA levels.

These findings suggest that the activation of GR genomic pathway is fundamental for the correct cognitive performance, while CMS exposure induces a behavioral deficit probably interfering with this mechanism. This inactivation of GR in stressed rats might be indicative of the so-called "glucocorticoid resistant" a key feature of depressed patients. Moreover, CMS, increasing the availability of GR at membrane levels, seems to direct preferentially the action of hormones to the non-genomic pathways.

P14.7
A SPECIFIC SOCIAL INTERACTION TEST FOR THE STUDY OF A MOUSE MODEL OF SOCIAL ANXIETY DISORDER

Boudjafad Z[1], Mobaligh M[1], Lamiri FZ[1], Bennis M[1], Garcia R[2] and Ba-M'hamed S[1]

[1] *Laboratoire de Pharmacologie, Neurobiologie et Comportement, Centre National de la recherche scientifique et technique, URAC 37, Université Cadi Ayyad, Marrakech, Morocco*

[2] *Institut de Neuroscience de la Timone, UMR7289, Université Aix-Marseille et Centre National de la Recherche Scientifique, 13385 Marseille, France*

Social anxiety disorder (SAD) is characterized by a marked and persistent fear about social situations including social interactions, performing in front of others and participating in grouped activities. Once, largely neglected by the medical community, SAD gained more attention a decade ago but remains poorly elucidated. Therefore, animal models of SAD have been established in order to study the underlying etiology. Nevertheless, social interaction tests used to assess SAD related behavioral deficits are not specific and are far to mimic human social situations. Indeed, social behaviors in rodents are often studied in pairs under artificial and restricted conditions where no collective social activities are created. Hence, our current study aimed to establish a novel social interaction test based on the evaluation of social behavior of a mouse put in a rich environment with a group of three unfamiliar con-specifics.

To achieve this goal, we utilized a mouse model of social fear conditioning (SFC) to induce SAD and assessed, one day after, the social interaction test through the evaluation of three main parameters: i) time spent

by the experimental mouse investigating arena objects when being close or away from unfamiliar con-specifics; ii) time spent exploring unfamiliar con-specifics and iii) freezing time.

Our results revealed that, one day after SFC, conditioned mice showed a significant increased individual object investigation compared with unconditioned mice which showed intensive collective object investigation. Moreover, conditioned mice showed an increased freezing time and a decreased social approach time compared with unconditioned mice.

To conclude, while most social interaction tests evaluated mainly social avoidance or social approach behaviors in the study of SAD, our test was able to provide a new experimental framework for studying social interactions in more complex situations close to those observed in humans.

P14.8
PAINT THINNER EXPOSURE ALTERS BEHAVIORS AND ADULT HIPPOCAMPAL NEUROGENESIS IN MICE

Malloul H[1], Bonzano S[2,3], Bennis M[1], De Marchis S[2,3], Ba-M'hamed S[1]

[1]*Laboratory of Pharmacology, Neurobiology and Behavior (URAC-37), University Cadi Ayyad, Faculty of Sciences Semlalia, Marrakech, Morocco*

[2]*Department of Life Sciences and Systems Biology, University of Torino, Italy*

[3]*Neuroscience Institute Cavalieri Ottolenghi (NICO), Orbassano, Italy*

Thinner is a highly toxic chemical solvent that has psychoactive properties when inhaled. Chronic thinner abuse causes several behavioral and functional abnormalities. However, the mechanisms involved in these effects are relatively poorly understood. Given the well-known critical role of adult hippocampal neurogenesis in learning and memory and its implication in disease conditions associated with cognitive impairment, depression, and anxiety, we investigated its possible alteration following thinner inhalation.

Tests evaluating anxiety, depression, learning and memory function were performed after acute, subchronic and chronic thinner inhalation. In addition, adult hippocampal neurogenesis was evaluated by using means immunohistochemical markers of neurogenesis.

Our results demonstrate that chronic exposure to thinner resulted in increased depression-like behaviours and an anxiolytic effect. Behavioral tests showed that while no deficits were found in acutely treated mice, significant alterations were found in both subchronic and chronic exposed mice, indicating that long term inhalant treatment impacts also on learning and memory. In addition, long term thinner-treatment, but not acute treatment, decreased the rate of neurogenesis in hippocampal dentate gyrus as shown by reduced number of proliferating progenitors and immature neurons in this structure.

The exposure to thinner affects hippocampal neurogenesis by impairing survival of newborn cells and reducing progenitor proliferation. On the whole, these findings support a possible causal link between adult neurogenesis alteration and behavioral dysfunction associated with thinner exposure.

P14.9
DIVERSE EFFECTS OF CODEINE MICRONIJECTIONS IN SOLITARY TRACT NUCLEUS AND LATERAL TEGMENTAL FIELD ON COUGH IN CATS

Simera M, Poliacek I, Veternik M, Kotmanova Z, Machac P and Jakus J

Institute of Medical Biophysics, Faculty of Medicine, Comenius University, Mala Hora 4, 037 54 Martin, Slovak Republic

Microinjections of codeine (3.3 mM or 33 mM) in the solitary tract nucleus rostral to the obex (rNTS), caudal to the obex (cNTS) and the medullary lateral tegmental field (FTL) were performed on 27 anesthetized spontaneously breathing cats. Tracheo-bronchial cough was induced by mechanical stimulation of the trachea with a soft nylon fiber. Blood pressure, esophageal pressure (EP) and electromyograms (EMGs) of the diaphragm (DIA) and abdominal muscles (ABD) were recorded.

Microinjections of 3.3 mM codeine in the rNTS (68 ± 4 nl for both microinjections, the total dose 0.22 ± 0.02 nmol, 6 cats) resulted in reduced cough number, expiratory amplitudes of ABD EMG and EP and amplitudes of DIA EMG during cough. There was no significant change in the duration of cough phases in response to microinjection of codeine into the rNTS. The only altered cough related time interval was the prolongation of the period between the peak activity of DIA and ABD (from 0.24 ± 0.01 s to 0.31 ± 0.03 s, $P < 0.05$).

Codeine microinjections into the cNTS (108 ± 33 nl for both microinjections, total dose 1.56 ± 0.58 nmol, 3 cats with 3.3 mM and 3 cats with 33 mM codeine) had no significant effect on tracheobronchial cough (all $P > 0.1$).

Microinjections of 33 mM codeine in the FTL (66 ± 6 nl for both microinjections, the total dose 2.18 ± 0.19 nmol, 7 cats) reduced cough related amplitudes of DIA and ABD EMGs, inspiratory and expiratory EP amplitudes. There was no significant effect of codeine microinjections into the FTL on number of coughs and the temporal characteristics of cough.

Control microinjections of a vehicle in all selected

areas had no significant effect on cough.

Our data showed that codeine has a diverse effect on cough reflex in these brainstem locations. Unlike the cNTS, the rNTS and the adjacent region of the reticular formation (rostral and dorsal FTL) contains codeine sensitive complex neuronal circuits involved in control of cough reflex in the cat.

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P14.10
REPEATED CORTICOSTERONE ADMINISTRATION ATTENUATES THE MODULATION OF GABA-ERGIC TRANSMISSION BY 5-HT$_7$ RECEPTOR IN THE RAT DORSAL RAPHE NUCLEUS

Sowa J[1], Kusek M[1], Tokarski K[1] and Hess G[1,2]
[1]*Institute of Pharmacology, Polish Academy of Sciences, Department of Physiology, 12 Smętna St., Kraków, Poland*
[2]*Institute of Zoology and Biomedical Research, Jagiellonian University, 9 Gronostajowa St., Kraków, Poland*

Chronic stress and elevated level of corticosterone have been implicated in the pathology of depressive disorders. Dorsal raphe nucleus (DRN), being a major source of brain serotonin, regulates the stress response and is involved in the development of stress-related psychiatric disorders. The 5-HT$_7$ receptor is one of several serotonin receptor subtypes expressed in the DRN, where it modulates GABA-ergic transmissionì. The aim of this study was to determine the effects of repeated corticosterone administration on GABA-ergic inputs to serotonergic neurons of the DRN and their modulation by the 5-HT$_7$ receptor.

Male Wistar rats received subcutaneous injections of corticosterone (10 mg/kg, volume 1 ml/kg) or the vehicle (1% Tween 80; volume 1 ml/kg) twice daily, for 7 or 14 days. The effects of these treatments were examined 24 h after the last administration. Whole-cell recordings were carried out from putative serotonergic neurons in slices containing DRN. To assess the GABA-ergic transmission spontaneous inhibitory postsynaptic currents (sIPSCs) were recorded. In some experiments 5-CT was applied in the presence of WAY 100635 to selectively activate the 5-HT$_7$ receptor.

In slices originating from rats treated with corticosterone for 7 as well as 14 days, the mean frequency, but not the mean amplitude of sIPSCs, was markedly lower than that in slices originating from control animals receiving vehicle. Activation of the 5-HT$_7$ receptor resulted in a significant increase in the mean frequency of sIPSCs in cells originating from control animals but not in corticosterone-treated groups.

These results suggest that corticosterone treatment causes an attenuation of the GABAergic transmission and affects the function of the 5-HT$_7$ receptor in the rat dorsal raphe nucleus. These effects may be related to stress-induced abnormalities in the functioning of the serotonergic system.

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P14.11
GENDER DIFFERENCES IN A NEW POTENT 5HT$_{2A}$ AGONIST EFFECTS: NEUROCHEMICAL AND BEHAVIORAL STUDIES AFTER 25I-NBOME ADMINISTRATION

Miliano C[1], Pintori N[1], Margiani G[1], Ossato A[3], Bilel S[3], Marti M[3] and De Luca MA[1,2]
[1]*Department of Biomedical Sciences, Neuropsychopharmacology Section, University of Cagliari, Italy*
[2]*National Institute of Neuroscience, University of Cagliari, Italy*
[3]*Department of Life Sciences and biotechnology (SveB), University of Ferrara, Italy*

25I-NBOMe, commonly called "N-Bomb", is a new synthetic compound, recently abused for its psychedelic and entactogenic effects; it is available on internet as a legal alternative to LSD, and as a surrogate of methamphetamine as well. It acts as full agonist of 5-HT2A receptor with high affinity on human and rat 5-HT$_{2A}$ receptors (Ki = 0.044 nM and Ki = 0.087 nM, respectively). Users are often unaware of ingesting fake LSD, and several intoxication cases and some fatalities have been reported after the ingestion. Overdoses of "N-Bomb" can cause several effects such as tachycardia, hypertension, seizures, and agitation persisting for up to three days. We decided to test 25I-NBOMe in both males and females to evaluate if there were gender differences in the pharmacological effects. In the current literature, there are no data about the abuse liability of this compound and its pharmacological effects.

By *in vivo* microdialysis studies, we evaluate the effects of 25I-NBOMe (0.3–1.0 mg/kg/ip) on dopamine (DA) and serotonine (5-HT) transmissions, both in male and female rats, moreover, sensorimotor studies, body temperature evaluation and nociception tests, were performed in both genders.

Our results showed that the phenethylamine 25I-NBOMe is more active in females, compared to males, in increasing DA transmission in NAc shell and in the mPFC; behavioral data showed that this compound caused visual alterations in both sexes, whereas core temperature in females is heavily affected, compared to males; indeed, the highest dose tested exerts an analgesic effect prominent in male rats, compared to female

rats.

Taken together these results suggest that 25I-NBOMe affects DA and 5-HT transmissions in male and females in a different way, highlighting gender differences that can influence the frequency of ingestion, as well as the psychoactive effects, and the long-term effects. Further investigations are necessary to examine in depth the reason of these gender differences.

P14.12
GENDER DIFFERENCES IN A NEW POTENT 5HT2A AGONIST EFFECTS: NEUROCHEMICAL AND BEHAVIORAL STUDIES AFTER 25I-NBOME ADMINISTRATION

Castro E[1,2], Ozoria C[1], Vasquez J[1] and **Pacheco-Herrero M**[1]

[1] *College of Medicine, Pontificia Universidad Católica Madre y Maestra (PUCMM), Autopista Duarte km* $1\frac{1}{2}$*, Santiago de los Caballeros, Dominican Republic*

[2] *College of Medicine, Universidad Jaime I, Av. De Vicent Sos Baynat s/n, Castellon de la Plana, Spain*

Alzheimer's disease (AD) is an insidious condition that represents the most common cause of dementia among elderly people. The brains of Alzheimer's patients have several distinctive neuropatological features: intracelular neurofibrillary tangles, senile plaques (SP) composed mainly of extracelular beta-amyloid (βA) and neurodegeneration. Due to the importance of the SP in the initiation and progression of neurodegeneration of AD, it has become one of the principal targets in therapeutic strategies against adverse neuronal outcomes. In this sense, nanotechnology is playing a decisive role in stopping or slowing the progression of this condition.

In this work, we characterize and review the peptide Cys-Leu-Pro-Phe-Phe-Asp (CLPFFD), capable of recognize and destabilize the SP, in vitro and *in vivo*, once adsorbed on magnetic nanoybrid, upon electromagnetic irradiation. For this goal, Raman Spectroscopy and Atomic Force Microscopy were performed.

Our data suggest that the use of magnetic nanohybrids attached with CLPFFD could be a good strategy for dissembling cerebral SP in the AD.

Additional experiments should be done to determine the effect of this approach in the reduction of the SP load and their implications in the memory of experimental models for this disease.

P14.13
IS THE COGNITIVE CONTROL IMPAIRED IN CHILDREN WITH AN ATTENTION DEFICIT/HYPERACTIVITY DISORDER?

Casini L

Aix-Marseille Université – CNRS, Laboratoire de Neurosciences Cognitives, Marseille, France

The Attention deficit/hyperactivity disorder (ADHD) is one of the most common developmental disorders diagnosed in childhood and it often persists into adulthood. It corresponds to symptoms of inattention, hyperactivity and impulsivity. These symptoms lead to great difficulties in school learning, and in social and familial relationships. A deficit in cognitive control is commonly found in children with ADHD. This has mainly been interpreted as due to difficulties in inhibiting inappropriate responses. However, cognitive control involves other processes than simply the ability to inhibit. Therefore, to deeper decipher cognitive control in children with ADHD, we used sophisticated analyses of the performance instead of classical measures such as mean reaction times (RT) and error rates. These analyses allowed us to dissociate the susceptibility of subjects to trigger an automatic response from their ability to suppress it. Moreover, we investigated the effect of methylphenidate (MPH) on these processes because MPH is the most prescribed medication and it is supposed to act on dopaminergic system.

We compared interference control between 25 children with ADHD without medication, 20 children with ADHD under MPH, and 20 control children performing a conflict task (the Simon reaction time task) well known to highly involve cognitive control. This task requires to inhibit an automatic response to the benefit of the action required by the rule.

Our data have shown that: 1) difficulties in cognitive control of ADHD children would be due to both a higher susceptibility to trigger automatic responses and an inhibition deficit, and 2) MPH improved this control by improving the selective inhibition of the automatic response without decreasing the strength of the automatic response. This suggests that these two processes would rely on different neurotransmitter systems, and more specifically, the selective inhibition only would rely on dopaminergic system.

P14.14
DETERMINATION OF GLUCOCORTICOIDS ACTION IN AN ANIMAL MODEL OF COEXISTENCE OF DEPRESSION AND OBESITY

Budziszewska B, Kurek A, Głombik K, Detka J and Basta-Kaim A

Department of Experimental Neuroendocrinology, Institute of Pharmacology, Polish Academy of Sciences, 12 Smetna Str, 31-343 Kraków, Poland

Epidemiological studies have indicated a frequent coexistence of depression and diabetes and similar changes in the structure and function of the central nervous system's cells are observed in animal models of both of

these disorders. Chronic stress, especially acting during prenatal period, is considered as a factor involved in pathogenesis of depression and diabetes and responsible for metabolic and morphologic changes occurring in these disorders. The aim of this study was to identify the markers which determine the potency of glucocorticoid action in the brain.

The study was conducted in adult male Sprague Dawley rats derived from mothers which were subjected to immobilization stress in the last week of pregnancy.

Animals, which underwent prenatal stress were divided, based on the results of the forced swimming test, into two groups: responsive and nonresponsive to stress. Half of the animals in control and stress groups received standard diet and the other high-fat diet. The concentration of corticosterone, glucocorticoid receptors (GR) and factors which regulate GR function - immunophilin FKBP-51and four translational Bag-1 isoforms were determined in the hippocampus and frontal cortex by ELISA and Western blot methods.

It has been found that none of the factors (prenatal stress, high-fat diet) affect the level of the GR in the hippocampus, while prenatal stress decreases the expression of the GR in the frontal cortex. There were no significant differences in the immunophilins FKBP-51concentrations in any of the examined brain structures. Among the studied Bag-1 isoforms, prenatal stress decreased level of Bag-1M, but not Bag-1L and Bag-1S, in the prefrontal cortex, whereas in the hippocampus prenatal stress and high-fat diet did not affect Bag-1 isoforms.

The obtained results indicate that reduction of Bag-1M concentration, a protein that inhibits the GR function and attenuates process of apoptosis, may increase glucocorticoids action and lead to cells damage in frontal cortex in prenatally stressed rats. Reduced level of GR in this structure can on the one hand, attenuate inhibitory mechanism of HPA axis regulation, but on the other hand may reduce direct, adverse effects of glucocorticoid.

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P14.16
CHARACTERIZATION OF SF-11, A SELECTIVE BRAIN PENETRANT ANTAGONIST OF THE NEUROPEPTIDE Y Y2 RECEPTOR: RAMAN SPECTROSCOPY AND BEHAVIORAL STUDIES

Domin H[1], Piergies N[2], Pięta E[2], Szewczyk B[1], Pochwat B[1], Oćwieja M[3], Paluszkiewicz C[2] and Śmiałowska M[1]

[1]*Institute of Pharmacology, Polish Academy of Sciences, Department of Neurobiology, 31-343 Kraków, Smętna Street 12, Poland*

[2]*Institute of Nuclear Physics Polish Academy of Sciences, PL-31342 Krakow, Poland*

[3]*J. Haber Institute of Catalysis and Surface Chemistry Polish Academy of Sciences, PL-30239 Krakow, Poland*

Recent studies have shown that Y2R antagonists, such as BIIE0246 and JNJ-31020028 produced antidepressant-like effects in a rat model of depression. The complex structure and high molecular weight of BIIE0246 limit its usefulness as an in vivo pharmacological tool. In this report, we described Raman (RS) and surface-enhanced Raman spectroscopy (SERS) studies of the structure of SF-11 [N-(4-ethoxyphenyl)-4-(hydroxydiphenylmethyl)-1-piperidinecarbothioamide], which is a novel, brain penetrant and low molecular weight Y2R antagonist. The knowledge of the molecular structure of SF-11 and its behavior at the solid/liquid interface is important for biomedical and biochemical research. The SERS spectra of SF-11 were recorded after its immobilization onto colloidal silver and gold nanoparticles. Moreover, the possible antidepressant-like activity of SF-11 was investigated. The drug was injected intraperitoneally into rats in a dose of 3 or 10 mg/kg and forced swim test (FST) was performed 1 h later.

The present SERS analysis was based on the observed changes in the position, enhancement, and broadening of the corresponding RS and SERS. For the colloidal silver SERS-active nanoparticles the significantly enhanced bands due to the para-substituted benzene ring and amine group vibrations, occurred. On the other hand, for the colloidal gold nanoparticles, the strong intensity bands related to the aromatic ring vibrations, were noticed. Our behavioral findings showed that SF-11 at a dose of 10 mg/kg, but not at a dose 3 mg/kg, produced a significant decrease in the immobility time in the FST in rats.

The obtained data indicate the SF-11 is adsorbed onto both SERS-active nanoparticles mainly through the benzylamine moiety. Moreover, the amine group strongly participates in the molecule/silver nanoparticles interaction. Our results also indicate that SF-11 exerts an antidepressant-like effect in rats and can be a useful tool for studying the role of Y2R in mood disorders.

P14.17
INSULON-LIKE GROWTH FACTOR-1 EXPRESSION IN CEREBELLUM OF DOGS INFECTED WITH CANINE DISTEMPER VIRUS

Yarim M and Karaca E

Department of Pathology, Faculty of Veterinary Medicine, Ondokuz Mayis University, Samsun, Turkey

Insulin-like growth factor-1 (IGF-1) is a neurotrophic factor that inhibits demyelination by protecting oligodendrocytes from damage and inhibiting their apoptosis. This study aims to investigate expression of IGF-1 in white matter of dogs cerebellum which has infected by canine distemper virus (CDV) and showing signs of demyelination. Paraffin blocks of 20 cerebellum naturally infected with CDV and 10 cerebellum of healthy dog were used in the study. CDV and IGF-1 expression levels were immunohistochemically determined in dog cerebellum sections.

IGF-1 expression was present in both control and CDV infected cerebellums but in CDV group, expression of IGF-1 was significantly higher in immunoreactive astrocytes. IGF-1 expression was not seen in the center of demiyelination areas while its expression was increased surrounding demiyelination areas. Our findings shows no correlation between IGF-1 expression and demyelination severity and CDV intensity.

As a result, it was considered that IGF-1 expression was increased around the sites of demyelination in CDV infection, which could be interpreted as an effort to prevent demyelination by reducing oligodendrocyte damage. From present findings it may considered that IGF-1 supplements may alleviate demyelinating diseases at the early stage by reducing oligodendrocyte damage and demyelination. This research was supported by Ondokuz Mayis University Research Fund (PYO.VET.1901.16.009).

P14.18
CEREBROSPINAL FLUID AND GENETIC BIOMARKERS IN EARLY DETECTION OF ALZHEIMER'S DISEASE

Babić Leko M[1], Nikolac Perković M[2], Langer Horvat L[1], Klepac N[3], Borovečki F[3], Hof PR[4], Pivac N[2] and Šimić G[1]

[1]*Department of Neuroscience, Croatian Institute for Brain Research, University of Zagreb School of Medicine, Zagreb, Croatia*

[2]*Ruđer Bošković Institute, Division of Molecular Medicine, Zagreb, Croatia*

[3]*Department for Functional Genomics, Center for Translational and Clinical Research, University of Zagreb Medical School, University Hospital Center Zagreb, Zagreb, Croatia*

[4]*Fishberg Department of Neuroscience and Friedman Brain Institute, Icahn School of Medicine at Mount Sinai, New York, USA*

Early diagnosis of Alzheimer's disease (AD) in asymptomatic individuals is crucial because potential therapeutics should be administrated as early as possible, when neurodegeneration is not yet advanced. In this study we assessed whether the diagnostic potential of cerebrospinal fluid (CSF) biomarkers amyloid $\beta_{1\text{-}42}$ ($A\beta_{1\text{-}42}$), total tau (t-tau), tau phosphorylated at epitope 181 (p-tau$_1$81), epitope 199 (p-tau$_1$99), epitope 231 (p-tau$_2$31) and visinin-like protein 1 (VILIP-1) could be improved by genetic biomarkers related to serotonin metabolism (5-HT_{2A}, 5-HT_{1B}, 5-HT_{2C}, and *MAOB*), inflammatory pathways (IL1α, IL1β, IL10, IL6, and TNFα), catecholamine metabolism (*COMT*, *DBH*), and survival of neurons (*BDNF*). We compared levels of $A\beta_{1\text{-}42}$, t-tau, p-tau_{181}, p-tau_{199}, p-tau_{231}, and VILIP-1 between patients with different *DBH* (rs1611115), IL1α (rs1800587), IL1β (rs1143623), IL6 (rs1800795), IL10 (rs1800896), TNFα (rs1800629), 5-HT_{2A} (rs6313), 5-HT_{1B} (re13212041), 5-HT_{2C} (rs3813929), *COMT* (rs4680), *BDNF* (rs6265), and *MAOB* (rs1799836) genotypes. The study was conducted on 115 AD and 53 mild cognitive impairment (MCI) patients, 10 healthy controls and 56 patients with other causes of dementia (14 with vascular dementia [VaD], 23 with frontotemporal dementia, 7 with dementia with Lewy bodies, 3 with AD + VaD, 1 with corticobasal degeneration, 1 with hydrocephalus, 2 with Parkinson's disease, 1 with epilepsy, and 4 with unspecified dementia). Levels of t-tau, p-tau_{181}, p-tau_{199}, p-tau_{231} and VILIP-1 were significantly higher in subjects with AA compared to GG and AG TNFα genotype (in patients with other dementias and in AD patients). Levels of p-tau_{199} and p-tau_{231} were significantly higher in patients with other dementias with CG compared to CC IL1β genotype. Levels of t-tau, p-tau_{181} and p-tau_{231} were significantly higher in patients with other dementias with AA compared to AG *COMT* genotype, while levels of $A\beta_{1\text{-}42}$ were significantly lower in AD patients with GG compared to AG *COMT* genotype. Levels of p-tau_{181} were significantly higher in patients with other dementias with TT compared to CC and TC IL10 genotype, while levels of p-tau_{199} and p-tau_{231} were significantly higher in AD patients with CC compared to TC IL10 genotype. Levels of VILIP-1 were significantly higher in MCI patients with GC compared to GG IL6 genotype, while levels of p-tau199 were significantly higher in MCI patients and levels of t-tau were significantly higher in patients with other dementias with GC compared to CC IL6 genotype. Levels of p-tau_{181} were significantly higher in AD patients with GA compared to AA and GG *BDNF* genotype and in MCI patients with CT compared to CC *DBH* genotype. Levels of $A\beta_{1\text{-}42}$ were significantly lower in MCI patients with AA compared to GG MAOB genotype. The potential of TNFα (rs1800629), IL1β (rs1143623), *COMT* (rs4680), IL10 (rs1800896), IL6 (rs1800795), *BDNF* (rs6265), *DBH* (rs1611115) and

MAOB (rs1799836) polymorphisms in early diagnosis of AD should be tested further and validated on larger cohorts of patients. This work was supported by Croatian Science Foundation grant IP-2014-09-9730.

P14.19
INHIBITION OF GLYCOGEN SYNTHASE KINASE-3 AS A PROTECTIVE STRATEGY AGAINST MUTANT HUNTINGTIN'S-INDUCED TOXICITY

Rippin I and Eldar-Finkelman H
Department of Human Molecular Genetics and Biochemistry, Sackler School of Medicine, Tel Aviv University, Israel

Glycogen synthase kinase-3 (hereafter GSK-3), a serine-threonine protein kinase with essential roles in diverse biological processes, has been identified as a therapeutic target for diverse neurodegenerative diseases. These diseases share common pathological characteristic of accumulation of aggregated neurotoxic proteins. This is often coupled with impaired autophagy and lysosome activity. We hypothesized that inhibition of GSK-3 may alleviate these toxic effects by balancing cellular clearance activity.

Our laboratory develops substrate competitive GSK-3 inhibitors. L807mts is a highly selective and potent GSK-3 inhibitor that functions as substrate-converting into an inhibitor.

In these studies we investigated if GSK-3 inhibition protects cells from mutant huntingtin-induced toxicity (mHtt), and whether mechanistic target of rapamycin complex-1 (mTORC1) and autophagic pathways are involved. We used SH-SY5Y cells that were transiently transfected with GFP-mHTTQ74 plasmid and express ploy-glutamine mHtt. GFP-mHtt aggregates were formed 48 hours post transfection, and treatment with Torin1 or L807mts reduced the content of these aggregates. Furthermore, Like Torin1, L807mts supported cell viability of cells expressing mHtt. Moreover, L807mts increased mTORC1 activity and levels of autophagy markers such as LC3-II, p62/SQSTM1 and beclin-1. We suggests that GSK-3 inhibition increases autophagic activity, that in turn, alleviates cellular stress induced by mHtt, activates mTORC1, and protects cells from mHtt toxicity and cell death.

P14.20
FITOHORMONE ABSCISIC ACID TREATMENT AMELIORATES NEUROINFLAMMATION AND COGNITIVE IMPAIRMENT INDUCED BY HIGH FAT DIET

Sánchez-Sarasúa S, Moustafa S, Garcia-Aviles A, Olucha-Bordonau FE and Sánchez-Pérez AM
Facultad de CC de la Salud, Universidad de Jaume I, Castellón de la Plana, (Castellón), Spain

Many neurological diseases have an inflammatory etiology and insulin resistance is a key factor in Alzheimer disease. Molecules inhibiting neuroinflammation might also be efficacious in the prevention and/or treatment of neurological disorders of inflammatory etiology. The abscisic acid (ABA) is the main phytohormone involved in abiotic stress responses. However, this compound is not only found in plants but also in other organisms, including bacteria, fungi and animals. Interestingly, it can be synthesized and secreted by a variety of human cells. Recent studies suggest a role of ABA regulating immune response and insulin action. Taking these data together we decided to ascertain if ABA has a protective effect in neuroinflammation, and brain insulin metabolism.

We chose a model of neuroinflammation that involved feeding the animals with a High Fat Diet (HFD), this model induces glucose resistance and an increase of proinflammatory markers in peripheral tissues. Experimental groups included, HDF alone; HFD with ABA; and control diet with and without ABA.

We confirmed that, in our model, ABA restores glucose tolerance in HFD rats, to levels of control diets rats' levels. Behavior paradigms show that HFD impairs lightly but significantly animal memory in T-maze but not in novel object recognition. Interestingly, ABA restores the cognitive performance of HFD fed animals to control levels. In hypothalamus and septum, ABA can restrain microglia increase induced by high fat diet. Moreover, ABA can curtail the number of cytokine and other insulin resistance and inflammatory markers in hypothalamus. The mRNA levels of IRS1 and IRS2 are reduced by HFD and ABA reverse lightly but not significantly this effect in hippocampus. These results suggest that ABA might become a new therapeutic molecule improving cognitive and metabolic processes associated to neuroinflammatory conditions and insulin resistance.

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P14.21
CLUSTER ANALYSIS OF BRAINSTEM NEURONAL DISCHARGE PATTERNS SUGGESTS THE EXISTENCE OF DISTINCT CLASSES OF BREATHING MODULATED FIRING RATES

Morris KF, Shuman HD, O'Connor R, Segers LS, Nuding SC, Horton K, Alencar PA, Bolser DC and Lindsey BG
University of South Florida, Morsani College of Medicine, Department of Molecular Pharmacology and

Physiology, USA

There has been no generally accepted quantitative description of the respiratory modulation of brainstem neuron firing rates. More significantly, the question remains whether there exist distinct classes of respiratory modulated neurons or rather they constitute a continuum. Building on previous work, we have developed a quantitative, reproducible clustering classification protocol to address these issues.

We surveyed historical recordings of extracellular potentials of spike trains in respiratory related brainstem regions, i.e. lateral respiratory column (ventral respiratory group, pre-Bötzinger, Bötzinger), pons, raphé, and dorsal lateral medulla including the dorsal respiratory group. We calculated 100-bin normalized cycle triggered histograms (CTH) of 1,373 spike trains. Ward clustering with Cartesian distance in 100-space (the normalized CTH bins) first differentiated the spike trains with significant respiratory modulation ($n = 573$) into three groups: predominantly inspiratory, predominantly expiratory and low respiratory modulation.

Application of the same clustering paradigm to each of these 3 groups yielded a set of 24 respiratory archetypes consisting of averaged histograms of the elements of each cluster. We used those archetypes as clustering centroids on naïve recordings of 229 spike trains; all were matched, i.e clustered, with 20 of the archetypes.

Preliminary analysis suggests the existence in brainstem neurons of distinct classes of respiratory modulation. Support: OT2OD023854-01, HL109025.

P14.22
CLUSTER ANALYSIS OF BRAINSTEM NEURONAL DISCHARGE PATTERNS SUGGESTS THE EXISTENCE OF DISTINCT CLASSES OF BREATHING MODULATED FIRING RATES

Tong Y[1,2] **and Khalil R**[1,2]

[1]*Center for Molecular and Behavioral Neuroscience, Rutgers University, Newark, USA*

[2]*Behavioral and Neural Sciences Graduate Program, Rutgers University, Newark, USA*

Could the balance act between excitation/ inhibition (E/I) within and across different cortical areas shape the cortical neuronal activity in normal and pathological conditions? Accumulated evidences showed the critical impact of balance act within the cortical areas in optimizing the cortical plasticity and functional connectivity. Subsequently, number of studies reported about the imbalance between excitation inhibition in certain neurological and psychiatric disorders such as autism spectrum disorders (ASDs) and schizophrenia. Now, the question is how this balance act could shape the neuronal activity across different cortical regions (i.e., the primary visual cortex (V1) and the prefrontal cortex (PFC)), and what goes wrong in case of pathological condition. Based on the current literatures, there are several evidences showing the critical impact of the balance act in V1 and PFC. However, implications of the balance act (E/I) between these two regions at the neuro-circuit level remain elusive. Therefore, we aim at highlighting this issue in normal and pathological condition focusing on the functional projection from V1 to PFC and vice versa in rodents.

We propose using integrated approach combining optogenetic, in vivo recording and behavioral tasks. The aim is to study the changes in excitation/inhibition balance locally (within V1 and PFC) and globally (across V1 and PFC) at the microcircuit level.

According to the previous findings, Parvalbumin (PV) neurons of the rodents but not somatostatin (SST) neurons are essential for establishing E/I balance. As for PV neurons, they exist in both V1 and PFC, and develop earlier in V1 in comparison to PFC during cortical development. Additionally, PV protein has higher expression in V1 in comparison to PFC in adolescence rodents. In terms of the distribution of PV neurons across layers in V1 and PFC, PV neurons reside layer II-VI of both areas though layer II/III of PFC, which has less PV neurons. In V1, PV neurons play a critical role in equalizing E/I balance by offset the excitatory neurons of layer IV. Nevertheless, this phenomenon has not been demonstrated in PFC. Interestingly, direct projections between V1 and PFC have been found recently. As for visual deprivation, it was suggested to rapidly reduce the number of PV interneurons, and thus disinhibit the pyramidal neurons of layer II/III (of V1). Furthermore, PV reduction in V1 induces a significant increase in the number of PV neurons in layer II/III, and layer V/VI of PFC.

Rodent's studies show that PV interneurons in V1 and PFC play a vital role in E/I balance. However, the interaction between these two areas is not investigated enough despite its critical neurological impact in health and disease.

In conclusion, we aim at shedding the lights on the ultimate need of establishing more research in this area (balance act between E/I between V1 and PFC) so that we can understand the diseased model better.

P14.23
PRESYNAPTIC N-METHYL-D-ASPARTATE RECEPTOR INHIBITS CA^{2+} CURRENTS AT A RAT CENTRAL GLUTAMATERGIC SYNAPSE

Oshima-Takago T[1,2] **and Takago H**[1,2,3]

[1]*Department of Rehabilitation for Sensory Functions, Research Institute, National Rehabilitation Center for Persons with Disabilities, Saitama, Japan*

[2]*Department of Neurophysiology, University of Tokyo Graduate School of Medicine, Tokyo, Japan*
[3]*Department of Otolaryngology, Tokyo Medical and Dental University Graduate School, Tokyo, Japan*

N-methyl-D-aspartate receptors (NMDARs) play diverse roles in synaptic transmission, synaptic plasticity, neuronal development, and neurological diseases. In addition to their postsynaptic expression, NMDARs are also expressed in presynaptic terminals at some central synapses, and their activation modulates transmitter release. However, the regulatory mechanisms of NMDAR-dependent synaptic transmission remain largely unknown.

In this study we performed whole-cell voltage-clamp recordings from giant presynaptic nerve terminals called the calyx of Held as well as postsynaptic medial nucleus of trapezoid body (MNTB) neurons in the rat auditory brainstem, and pharmacologically examined the property and mechanism of presynaptic NMDAR-dependent synaptic modulation. Activation of NMDARs in nerve terminals at the calyx of Held synapse inhibits presynaptic Ca^{2+} currents (I_{Ca}) by means of GluN2C/2D subunit-dependent manner, thereby suppressing nerve-evoked glutamate release. Neither presynaptically-loaded fast Ca^{2+} chelator BAPTA nor non-hydrolyzable GTP analogue GTPγS affected the NMDAR-mediated I_{Ca} inhibition. Repetitive activation of I_{Ca} in the presence of a glutamate uptake blocker attenuated the decline of I_{Ca} amplitude, suggesting that endogenous glutamate has a potential to activate presynaptic NMDARs.

We conclude that presynaptic NMDARs can attenuate glutamate release by inhibiting voltage-gated Ca^{2+} channels at a central glutamatergic synapse in the rat brainstem.

P14.24
INHIBITORY EFFECT OF RXFP3 ACTIVATION ON MAGNOCELLULAR PVN NEURONS *IN VITRO* - A POTENTIAL NEURONAL MECHANISM OF OREXIGENIC RELAXIN-3 ACTION IN MALE AND FEMALE RATS

Kania A[1], Szlaga A[1], Sambak P[1], Guguła A[1], Hess G[1], L. Gundlach A[2] and Blasiak A[1]
[1]*Department of Neurophysiology and Chronobiology, Jagiellonian University, Krakow, Poland*
[2]*The Florey Institute of Neuroscience and Mental Health, Melbourne, Australia*

Relaxin-3 is a stress-responsive, orexigenic peptide present in nucleus incertus neurons and their projections throughout the rat forebrain, including the hypothalamus. The hypothalamic paraventricular nucleus (PVN) is an established site for the induction of feeding following central relaxin-3 administration. Notably, relaxin-3 produces stronger orexigenic effects in female than male rats, but the neural mechanisms underlying this difference are unknown. Our *in vitro* studies in male rats revealed that relaxin-3 receptor (RXFP3) activation inhibits PVN magnocellular neuron activity. The aim of the current study was to investigate possible differences in the effects of, or sensitivity to, RXFP3 activation in PVN neurons from male and female rats.

Whole-cell, patch clamp recordings in current- and voltage-clamp mode were performed on hypothalamic slices from male and female Sprague-Dawley rats (6-8-week old) to assess possible changes in membrane potential, whole cell current and synaptic transmission elicited by RXFP3 activation. All drugs were applied via bath perfusion.

In current-clamp experiments, the RXFP3-selective agonist RXFP3-A2 (600 nM) decreased the spontaneous firing frequency of the majority of PVN neurons recorded in both sexes (by 2.2 ± 2.4 Hz, $p < 0.05$, $n = 11$, in males; and by 2.0 ± 1.7 Hz, $p < 0.01$, $n = 12$, in females; with no significant difference in response between sexes, $p = 0.78$). The effect persisted in the presence of TTX and glutamate and GABA receptor antagonists, leading to membrane hyperpolarisation of 2.6 ± 1.5 mV, $p < 0.01$, in males; and 2.7 ± 1.5 mV, $p < 0.001$, in females; with no significant difference in response between sexes, $p = 0.88$). In voltage-clamp experiments RXFP3-A2 (600 nM) application increased outward whole cell current by 30.3 ± 14.3 pA, $p < 0.0001$, $n = 11$, in males; and by $28. \pm 16.1$ pA, $p < 0.0001$, $n = 12$, in females; with no difference in response amplitude between sexes, $p = 0.78$). No significant differences were observed in the baseline spontaneous firing frequency or whole cell current in the male and female magnocellular PVN neurons recorded.

Our data indicate that an inhibitory influence of relaxin-3/RXFP3 signalling on magnocellular PVN neurons is associated with feeding control in both sexes. Current studies are aimed at investigating potential sex differences in electrophysiological properties of magnocellular PVN neurons as well as in signalling pathways and ionic mechanism activated by RXFP3 in male and female rats.

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P15.1
EFFECT OF CERVICAL HEMISECTION ON SWALLOW AND AIRWAY PROTECTION

Huff A, Greene C, Cheffer K, O'Steen W, Howland D and Pitts T
Department of Physiology, Kentucky Spinal Cord Injury Research Center, University of Louisville, KY, USA

The effects of cervical hemisection on swallow have not been determined. We hypothesized that cervical hemisection would increase swallow excitability and shift the pattern of swallow breathing coordination to maintain pharyngeal clearance. Electromyograms of the mylohyoid, geniohyoid, thyrohyoid, thyroarytenoid, thyropharyngeus, cricopharyngeus and diaphragm (costal and crural) muscles were recorded in anesthetized, spontaneously breathing anesthetized cats prior to and after a C2 hemisection. Swallow was elicited by infusion of 3ccs of water into the oropharynx. Acute C2 cervical hemisection significantly increased EMG amplitudes across all upper airway muscles during swallow, and swallow frequency increased from 3.3 ± 1.2 to 8 ± 1.4 per infusion. Significant changes in swallow-breathing coordination were noted with all swallows occurring in E1 (as opposed to late E2), significantly increasing the risk for potential aspiration. These results support a theory of spinal cord inhibition/modulation of the swallow pattern generator and upper airway muscle excitability, as well as the importance of its role in swallow/breathing integration. Supported by R00- HL 111215, The Kentucky Spinal Cord and Head injury Trust, The Commonwealth of Kentucky Challenge for Excellence, the Rebecca F Hammond Trust and RCS-VA RR&D B9249S. The contents of this abstract do not represent the views of the DVA or US government.

P15.2
MAPPING CFOS EXPRESSION AFTER CEREBELLAR AND MEDIAL PREFRONTAL DEACTIVATIONS IN RATS TRAINED TO ACQUIRE COCAINE-INDUCED PREFERENCE CONDITIONING

Gil-Miravet I, Guarque-Chabrera J, Musoles-Lleo JL, Olucha-Bordonau F and Miquel M
Universitat Jaume I, Castellon, Spain

Pavlovian memories of preference for drug-related stimuli are crucial components to drive motivational trigger of drug seeking and drug taking behaviours. Despite growing data in the last years, the cerebellum has remained excluded from the circuitry sustaining these behaviours. However, the cerebellum presents close anatomical and functional connectivity in several key regions in the striatum-cortico-limbic circuitry. Recently, we have found two cerebellar hallmark signatures of conditioned preference for cocaine: an increase in cFos expression in cells at the apex of the granule cell layer and a strong expression of the perineuronal nets in the same region of the cerebellar vermis. In the present investigation, we evaluated the effects of different medial prefrontal cortex (mPFC) and cerebellar deactivations in rats before starting with the conditioning training to acquire preference for an olfactory stimulus paired with cocaine. Two groups of rats were subjected before training to a temporary prelimbic or infralimbic inactivation by lidocaine. Another two groups were treated with quinolinic acid for a permanent lesion in the dorsal or ventral area of lobule VIII in the cerebellar vermis. Sham control rats received vehicle in the same regions. cFos expression was evaluated in different areas of the striatum-cortico-limbic circuitry to analyse changes in activity patterns after these brain interventions. The inactivation of infralimbic cortex or the lesion of the dorsal cerebellar vermis promoted the acquisition of cocaine-induced preference conditioning. Interestingly, the combined lesions of both areas regions prevented the facilitation of this conditioned response. Opposite results were found after either prelimbic deactivation or ventral cerebellar lesion. The change in cFos expression patterns was restricted to specific regions of amygdala and thalamic complex after dorsal cerebellar lesion. In this case, cFos expression increased significantly. Also, neural activity in either infralimbic cortex or cerebellum was enhanced after deactivations of each of these distal sides. The results suggest that the infralimbic cortex and dorsal posterior cerebellum work together and take part in the circuit that allow the inhibitory control of drug-related emotional memories.

P15.3
SERUM AND PLASMA LEVELS OF MATURE AND PRO-BDNF IN HEALTHY, AND EARLY MCI AND AD SUBJECTS OF VARIOUS AGES. PRELIMINARY CLINICAL SCREENING DATA

Pietrzkowski ZJ[1], Nemzer B[2], Cervantes M[1], Argumedo R[1], Hunter JM[2], Pond H[2] and Reyes-Izquierdo T[1]
[1] *Futureceuticals, Inc. 23 Peters Canyon Rd, Irvine CA 92606, USA*
[2] *Futureceuticals, Inc. 2692 N. State Rt. 1-17, Momence, IL 60954, USA*

The primary objective of this study was to investigate and potentially establish typical or average range levels of mature circulating BDNF in serum and plasma collected from: a) healthy subjects with age ranges from 25–35, 45–55 and 55–65; and, b) in people experiencing early symptoms of mild cognitive impairment (MCI) or Alzheimer's disease (AD) within age range of 55–65. Our secondary objective was to measure amounts of BDNF detected in circulating exosomes isolated from serum.

Serum and plasma samples were collected from 50 subjects within each age group and condition, for a total of 250 people. Serum collection and exosomes isolation were regularly performed in the morning after subjects had fasted at least 10 hours.

Collected data on serum and exosomal BDNF was correlated with age and health conditions in order to establish possible "healthy" BDNF ranges in young subjects, as well as potentially providing comparison criteria between healthy and unhealthy subjects. Analysis of exosomal BDNF levels supplied additional information related to the ratio between free circulating BDNF and exosomal BDNF that may pass BBB.

Preliminary analysis showed that levels of circulating BDNF (free and exosomal) are seem to be related to age and conditions. Analysis of serum-collected exosomes may provide more specific information about blood cells and their role in the release of exosomal BDNF. Preliminary results of these analyses are herein presented.

P15.4
A CRITICAL ROLE OF CA1 ACTIVITY FOR TEMPORAL BINDING

Azza S[1,2], Valério S[3], Al Abed SA[2], Oulé M[2], Lamothe V[2], Herry C[3], Potier M[2] and Marighetto A[2]

[1]*Faculté des Sciences de Tunis, Neurophysiologie fonctionnelle et pathologies, Tunis 2092, Tunisia*

[2]*Inserm u862, Neurocentre Magendie, Pathophysiology of Declarative Memory Group, Bordeaux-F33077, France*

[3]*Inserm u862, Neurocentre Magendie, Neuronal circuits of associative learning Group, Bordeaux-F33077, France*

Cognitive aging involves the prominent deterioration of declarative memory. Development of therapeutic approaches to cognitive aging may improve identifying processing mechanisms underlying this memory and their neurobiological bases. Previous work identified temporal binding, the capability to associate temporally distant stimuli, as a critical process for remembering complex associations among events, i.e. declarative memory. The CA1 subfield of the hippocampus is involved in memory of temporal associations, but its function remains to be specified. Based on the discovery of time cells, which fire at successive moments in temporally structured experiences, an hypothesis was recently proposed: activity of CA1 cells would bridge the gap in memory for discontiguous events.

To test this hypothesis, first, we confirmed that CA1 activity is related to successful temporal binding in memory by combining trace fear conditioning procedure at different levels of temporal binding demand (tone-shock interval length) to Fos neuroimaging. We found that successful tone-shock binding in memory only occurs for a tone-shock interval of 20 seconds and that CA1 is the sole area in which activity is increased by successful temporal binding compared to the other learning conditions under which no temporal binding occurs. Then we demonstrated that CA1 activity is necessary during temporal gaps between the (to be-associated) stimuli for successful temporal binding by combining trace fear conditioning to an optogenetic approach. We inhibited CA1 activity specifically in or out of the trace interval during acquisition of 20 seconds trace conditioning. We found that successful tone-shock binding in memory requires CA1 activity during temporal gap between the tone and the shock. In conclusion, our findings validate the "time cells" hypothesis that CA1 activity is critically needed during learning to bridge temporal gaps between discontiguous events in memory.

P15.5
BOOSTING PERCEPTUAL LEARNING WITH TRANSCRANIAL RANDOM NOISE STIMULATION RESULTS IN MORE EFFECTIVE VISUAL FUNCTION IMPROVEMENTS IN ADULTS WITH AMBLYOPIA

Moret B, Gorrieri R, Lo Giudice G, Veronese A, Rizzo R, Pavan A, Donato R and Campana G

University of Padova, Padova, Italy

Amblyopia is a neuro-developmental disorder characterized by several functional impairments in spatial vision (even with the best optical correction) in absence of any organic defects of the eye. Several studies have shown that extensive visual perceptual training can improve visual acuity (VA) and the contrast sensitivity function (CSF) in people with amblyopia, even in adulthood. With the present study we assess if the application of a high-frequency transcranial random noise stimulation (hf-tRNS) concurrently with a short perceptual training on adults with anisometric amblyopia is more efficacious in improving visual functions than the same perceptual training with Sham stimulation. Twenty participants were recruited and divided into two different groups: the experimental group underwent a short (8 sessions) contrast-detection monocular training with concurrent hf-tRNS while the control group underwent the same training with Sham stimulation. Results showed that, while a significant and clinically relevant improvement in VA and CSF occurred in the experimental group (mean VA improvement in the amblyopic eye was 0.18 LogMAR), no significant improvement in VA was seen for the control group.

Thus, in comparison with previous studies where a large number of sessions with a similar training regime was used, here an improvement of CSF has been found in both groups in just eight sessions of training, furthermore by adding hf-tRNS we have been able to transfer the enhancement to VA, a fuction not trained.

Our results support the idea that, by boosting the rate of perceptual learning via the modulation of neuronal

plasticity, hf-tRNS can be successfully used to reduce the duration of perceptual trainings and, at the same time, to increase generalization of perceptual learning to other nonpracticed visual function such as VA in patients with amblyopia.

P15.6
APPARENT EMOTIONAL EXPRESSION PREDICTS PERCEIVED TRUSTWORTHINESS WITH CHANGES OF HEAD POSTURE

Zhang D
Dalian University of Technology, China

People make trustworthiness judgments on the basis of facial cues rapidly and with high consensus. Emotional expressivity, as trait cues that humans use, plays an important role in detecting trustworthiness. Neuroimaging studies have provided evidence that trustworthiness judgements are linked to emotional expressions and that trustworthiness decisions are associated with activation of brain areas, such as the amygdala, that generally process emotional information. Changes in emotional expressions are likely to affect perceived trustworthiness but the effects of variation in head posture on apparent trustworthiness are not known.

Our studies therefore examined how head posture (level, up, or down) affects perceptions of trustworthiness. In Study 1, participants rated faces in three postures for apparent trustworthiness on seven-point Likert scales. In Study 2, participants scrolled through face images and manually manipulated vertical head angle to maximise perceived trustworthiness.

Results of ratings reveal that the head down posture decreased perceived trustworthiness compared to the level and raised head; the head up posture was perceived as less trustworthiness compared to the neutral posture. The optimal head angle to make the facial images most trustworthy was found to be slightly lowered with respect to the level posture. This posture made the facial expression appear more positive.

Our results suggest a profound effect of posture on apparent trustworthiness with a change in head posture. Our analysis reveals that apparent emotional expression provides an explanation of perceived trustworthiness. Together with recent neuroimaging evidence of brain localization of non-verbal cues processing, these findings highlight the functioning of head postures as social signal in social interaction.

P15.7
POTENT INHIBITION OF HUMAN CYP3A4 BY THE NOVEL ATYPICAL ANTIPSYCHOTIC DRUG ILOPERIDONE

Wójcikowski J, Danek P and Daniel WA
Polish Academy of Sciences, Institute of Pharmacology, Kraków, Poland

Inhibition of cytochrome P450 (CYP) isoenzymes in the liver and brain is the most common cause of harmful drug-drug interactions. Iloperidone is a novel atypical antipsychotic drug approved for the acute treatment of schizophrenia in adults. The aim of the present study was to evaluate the inhibitory effect of iloperidone on the main human CYP isoenzymes involved in the metabolism of psychotropic drugs.

Experiments were performed *in vitro* using pooled human liver microsomes. CYP isoform activities were determined using the following CYP-specific reactions: caffeine 3-N-demethylation (CYP1A2), diclofenac 4′-hydroxylation (CYP2C9), perazine N-demethylation (CYP2C19), bufuralol 4′-hydroxylation (CYP2D6) and testosterone 6β-hydroxylation (CYP3A4). The rates of CYP-specific reactions were measured by HPLC in the absence and presence of iloperidone (0.01–50 µM). Inhibition constants (Ki) for the inhibition of CYP-specific reactions by iloperidone were obtained using a non-linear regression analysis (Program Sigma Plot 8.0; Enzyme Kinetics). The obtained results showed that iloperidone potently inhibited CYP3A4 (Ki = 2 µM) and weakly diminished the activities of CYP1A2 and CYP2D6 (Ki = 64 and 98 µM, respectively). On the other hand, iloperidone did not affect the activities of CYP2C9 and CYP2C19.The presented findings may have significant implications for the prediction of potential interactions involving iloperidone and CYP3A4 substrates (e.g. antidepressants, benzodiazepines, calcium channel antagonists). Since the metabolic interactions found in the liver may also occur in the brain, the influence of iloperidone on brain CYP3A4 activity may be important for the metabolism of endogenous neuroactive substrates (e.g. neurosteroids), and for the local biotransformation of psychotropics: such interactions may modify their pharmacological action.

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P15.8
HDAC5 IMPAIRMENT IN FEAR MEMORY DEFICITS IN A NEURODEVELOPMENTAL MODEL OF SCHIZOPHRENIA

Latusz J, Bator E, Głowacka U and Maćkowiak M
Institute of Pharmacology, Polish Academy of Sciences, Laboratory of Pharmacology and Brain Biostructure 12 Smętna Str, 31-343 Kraków, Poland

Several findings indicate that deficits in emotional memory, i.e. fear memory might be related to impairment in epigenetic mechanism, i.e. histone H3 acetyla-

tion. Our previous study demonstrated fear memory deficits in trace fear conditioning (TFC) task induced by postnatal administration of CGP37849, a competitive antagonist of NMDA receptors. In the present study, we investigated whether the fear memory deficit in CGP-treated rats might be related to impairment in the regulation of histone H3 acetylation in the adult mPFC. To accomplish this task, we determined the level of histone deacetylase 5 (HDAC5) and its phosphorylated form pHDAC5 as well histone H3 acetylation at lysine 9 (H3K9ac) during memory retrieval in TFC. Immunoreactivity of HDAC5, pHDAC5 and H3K9ac were analyzed by western blot in nuclear and cytosolic fractions of the mPFC. We also examined the impact of the non-selective histone deacetylase inhibitor, sodium butyrate (SB) on memory retrieval and the level of epigenetic markers in TFC task. Postnatal blockade of NMDA receptor induced the increase in HDAC5 in nuclear fraction and the decrease in pHDAC5 in cytosolic fraction of the adult mPFC during memory retrieval. At the same time the decrease in H3K9ac was observed in nuclear fraction of the mPFC. In addition, SB administered 2 h after memory acquisition prevented the deficit in fear memory induced by postnatal CGP administration. Furthermore, SB also inhibited alterations in the level of HDAC5, pHDAC5 and H3K9ac in CGP treated animals.The obtain results indicate that postnatal blockade of NMDA receptor altered HDAC5 regulation by impairing phosphorylation-dependent nuclear export of HDAC5 and also affected histone H3 acetylation in the mPFC. The above impairment in epigenetic regulation might suppress expression of genes involved in memory formation and evoke fear memory deficits.

P15.9
IDENTIFYING THE INTERACTION BETWEEN CHROMOGRANIN A/PHOSPHATIDIC ACID AT THE LEVEL OF TGN MEMBRANE TO ELUCIDATE ITS ROLE IN THE BIOGENESIS OF SECRETORY GRANULES IN NEUROENDOCRINE CELLS

Montero-Hadjadje M[1], Carmon O[1], Delestre-Delacour C[1], Laguerre F[1], Tahouly T[2], Fouillen L[3], Renard PY[4], Vitale N[2] and Anouar Y[1]

[1] *Inserm U1239, University of Rouen Normandy, Mont-Saint-Aignan, France*

[2] *CNRS UPR 3212, INCI, Strasbourg, France*

[3] *CNRS UMR 5200, Plateforme Métabolome, Bordeaux, France*

[4] *CNRS UMR 6014 COBRA, Mont-Saint-Aignan, France*

Neuroendocrine cells are specialized in the secretion of neurohormones through the biogenesis of dense core-secretory granules (DCSG). These organelles bud from the TGN membrane after the interaction of neurohormone aggregates induced by soluble glycoproteins called chromogranins. Since the main member of this family, chromogranin A (CgA), acts as an on/off switch regulating the formation of DCSG, we decided to study the interaction between CgA and membrane lipids to highlight the molecular mechanisms mediating this process. Using lipid-protein overlay assays, we observed that recombinant CgA specifically binds to phosphatidic acid (PA). Phosphatidic acid being known as a crucial actor in the formation of secretory granules and in the process of membrane curvature, we are currently studying the interaction between CgA and phosphatidic acid in neuroendocrine cells and its role in the regulation of hormone secretion. The quantitative and comparative analysis by LC-MS/MS of the membrane lipidome of purified CgA granules and Golgi apparatus revealed an enrichment of PA in the granule membrane, and the predominance of PA36:1, PA38:2 and PA40:6 species. Moreover, using a pull-down assay with liposomes enriched with various phospholipids including phosphatidylserine, phosphatidylcholine or distinct PA species, we showed that CgA from cell lysate specifically interacts with the predominant PA species identified by the lipidome study. We postulate that CgA interaction with PA at the level of the TGN membrane is at the origin of microdomain formation that could govern the TGN membrane curvature and/or the recruitment of cytosolic proteins involved in the DCSG trafficking crucial to neurohormone secretion. To study these phenomena in living cells, we are currently working with organic chemists on the synthesis of biocompatible and photoactivatable PA analogues, and with biophysicians on the analysis of CgA/PA interaction-induced microdomains using of Fluorescence Correlative Spectroscopy.

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P15.10
INTERACTION OF GLUTAMATERGIC AND CHOLINERGIC TRANSMISSION IN ANIMAL MODELS OF SCHIZOPHRENIA

Cieślik P[1], Woźniak M[1], Archer F[2], Pilc A[1], Wierońska JM[1]

[1] *Institute of Pharmacology, Polish Academy of Sciences, Kraków, Poland*

[2] *Université Paris Descartes, Paris, France*

Schizophrenia is a severe mental disorder that is characterized by the prevalence of positive (hallucinations, delusions), negative (flattened affect) and cognitive (memory and attention deficits) symptoms. Unfor-

tunately, currently available drugs induce many adverse effects and poorly affect negative and cognitive symptoms. Recent data suggests that disruption in dopaminergic, glutamatergic, but also cholinergic transmission may underlie the pathology of schizophrenia. Our previous experiments show that positive allosteric modulation of muscarinic M_4 receptors has clear antipsychotic effect in animal models of schizophrenia. Thus, the aim of this study was to evaluate whether mGlu4 receptors are involved in antipsychotic activity of VU152100.

Male Albino Swiss mice were used in all behavioral tests. MK-801-induced hyperactivity, DOI-induced head twitches, modified forced swim test, social interaction test and novel object recognition test were used to assess the potential interaction between $mGlu_4$ and M_4 receptors in the context of antipsychotic activity.

Simultaneous administration of sub-effective doses of LSP4-2022 (selective mGluR4 agonist) and VU152100 (positive allosteric modulator of M_4 muscarinic receptors) reduced the MK-801-induced hyperactivity, however it had no effect on the number of head twitches. Co-administration of both substances increased the time and the number of social episodes in social interaction test and reduced the immobility time in modified forced swim test. Moreover, it reversed the MK-801-induced deficits in novel object recognition test.

Our results indicate that mGlu4-M_4 interaction can be beneficial in animal models of positive, negative and cognitive symptoms of schizophrenia. This suggests that concomitant administration of ineffective doses of substances acting on aforementioned receptors could provide an efficacious and safe alternative in treatment of schizophrenic patients as it could potentially minimalize adverse effects.

P15.11
THE PUTATIVE ANTIPSYCHOTIC-LIKE EFFECTS OF MGLUR7 RECEPTOR LIGANDS

Woźniak M, Cieślik P, Kaczorowska K, Pilc A and Wierońska JM

Institute of Pharmacology, Polish Academy of Sciences, Krakow, Institute of Nuclear Physics, Polish Academy of Sciences, Krakow, Poland

Variety of the previous research showed that the role of glutamatergic system in the pathology of schizophrenia plays very important role. As the data concerning the role of the IIIrd group of metabotropic glutamate receptors (mGluR) is rather scarce and considers mainly mGlu4 receptors ligands. The main interest of the present study was to investigate the role of mGluR7 receptor in animal models in schizophrenia.

In our research, we examined the effects of two mGluR7 ligands: selective, allosteric antagonist – MMPIP and negative allosteric modulator – ADX-71743. We conducted behavioral tests commonly used in antipsychotic drug discovery, such as MK-801-induced hyperactivity, DOI-induced head twitches, modified forced swim test, social interaction test and novel object recognition test. Both tested compounds dose-dependently inhibited MK-801-induced hyperactivity and DOI-induced head twitches. Moreover, the same effects we noticed in novel object recognition test, where MMPIP and ADX-71743 reversed MK-801-induced disturbances. However, the efficacy of drugs in the social interaction test was observed only for positive allosteric modulator of mGlu7 receptor, ADX-71743.

The present studies showed that mGlu7 receptor ligands may be considered as a target for antipsychotic drug discovery. However, due to limited number of available ligands more studies are needed.

P15.12
EXTINCTION FAILURE IN VULNERABLE TRAUMATIZED MICE

Lguensat A[1], Bentefour Y[1], Bennis M[1], Ba-M'hamed S[1] and Garcia R[2]

[1] *Laboratoire de Pharmacologie, Neurobiologie et Comportement, Centre National de la recherche scientifique et technique, URAC 37, Université Cadi Ayyad, Marrakech, Morocco*

[2] *Institut de Neuroscience de la Timone, UMR7289, Université Aix-Marseille et Centre National de la Recherche Scientifique, 13385 Marseille, France*

Post traumatic stress disorder (PTSD) is a debilitating disease triggered by the exposure to one or many traumatic events. Epidemiological studies indicate that PTSD prevalence can reach 23% to 35% in highly traumatized populations. Besides, clinical studies report that patients with PTSD have difficulties in fear extinction acquisition. Even though the traumatic event has the same intensity, the probability of developing PTSD following a similar level of exposure varies across individuals. Many animal models havebeen designed to study PTSD, but in most of them, the data collection and analysis are generally expressed as a function of exposed vs. non-exposed populations, regardless of individual variation in response.

Using an animal model of PTSD previously developed by our team, we individually examined the performance of animals in three behavioral tests (avoidance, fear sensitization and the elevated plus maze tests). An animal is labeled as "susceptible" to develop PTSD-like symptoms if its performance is more than one standard deviation from the mean of the control group in at least 2 tests. Concerning fear extinction, we re-exposed each animal to the trauma-associated context during 5 sessions. An animal is considered having difficulties in extinction learning if its performance is more than one

standard deviation from the mean of the control group in at least 3 extinction sessions.

In this context, our data revealed two subgroups of animals: PTSD-like susceptible subgroup with 34.6% of mice, and PTSD-like resilient subgroup with 65.4% of mice. Some of the mice in the PTSD-like susceptible subgroup were also characterized by poor extinction (higher scores during the first two extinction sessions).

These results indicate that vulnerable traumatized mice in our study expressed the most common features of PTSD. Our model seems therefore more relevant for animal PTSD studies.

P15.13
A ZONE OF CONNECTIVE SIMILARITY CONVERGENCE WITHIN THE TEMPORAL LOBE

Bajada CJ
Neuroscience and Aphasia Research Unit (NARU), School of Biological Sciences, The University of Manchester, UK

The temporal lobe underpins functions such as audition, olfaction and vision, to higher level functions such as speech perception culminating with the high level integrative function of semantics. A fundamental assumption of brain function is that it is dependent on the underlying structure of the brain and its connections. This project aims to identify, using diffusion magnetic resonance tractography, the area that demonstrates greatest connective similarity to all other areas and hence in an optimal location to perform integrative functions such as semantics. 24 healthy, right handed participants (11 F) were recruited. Diffusion weighted MRI images were acquired in 61 no-collinear directions. A T1 weighted image was acquired for visualisation purposes. Tractography was carried out using the probability index of connectivity tool. Unconstrained tracking was performed from every voxel in the temporal lobe giving each voxel a connectivity profile that can be represented as an ordered vector of connectivity values. The matrix of pairwise connective similarity between voxels was then computed as the cosine of the angle between the profile vectors of every pair of voxels. A spectral embedding algorithm was used to warp voxels into a connectivity space. Each voxel was given an RGB colour value that denoted their location in the connectivity space. The centre of mass of the cloud of voxel positions in the connectivity space was computed to identify the voxels that had connectivity profiles that were most similar to all other voxel profiles. Sharp connectivity boundaries were found between the medial temporal lobe and the lateral temporal lobe. Graded transitions were found within the lateral temporal lobe. The area which demonstrated connectivity profiles that were maximally similar to all other regions was on the anterior end of the ventral temporal lobe. The location of the convergence of connective similarity corresponds to regions known to be involved in semantic cognition. This lends support to the hypothesis that areas of the brain that have connectivity profiles that are maximally close to all other areas in a region may perform integrative functions such as semantics.

This work supports the idea that the anterior temporal lobe is an integrative hub.

P15.14
EFFECT OF PHOSPHODIESTRASE 10A INHIBITOR, MP-10 COMPOUND IN THE RAT IOWA GAMBLING TASK

Rafa D, Popik P and Nikiforuk A
Department of Behavioral Neuroscience and Drug Development, Institute of Pharmacology, Polish Academy of Sciences, 12 Smętna Street, 31-343 Kraków, Poland

Pathological gambling (PG) is a form of behavioural addiction. Both substance and behavioural addictions are characterized by the impairment in decision-making processes and impulsive responding. These components of PG can be investigated in a rat Iowa Gambling Task (rIGT). MP-10 is an inhibitor of phosphodiestrase 10A (PDE10A). These novel principles increase the level of cAMP and cGMP in the medium spiny neurons of the striatum and resemble the neurochemical consequences of dopamine D2 receptor inhibition and dopamine D1 receptor stimulation. Because it is postulated that dopaminergic systems are implicated in decision-making processes, including risky/safe choices, we investigated whether MP-10 could affect gambling behaviour in rats. We employed a novel model of PG in rodents, called the rat Iowa Gambling Task. In this task, the rats are trained in the Skinner boxes. The animals choose among four nose-poke holes, which differed in the amount of reward they provide, and in the probability and duration of punishing time-out periods, during which the reward cannot be earned. Subjects were trained to earn as many sugar pellets as possible within 30 min. After reaching a stable baseline, the test was performed. MP-10 was administered at doses 0.03, 0.10 and 0.30 mg/kg, P.O., 120 minutes before the test.

We report that MP-10 did not influence gambling behaviour at any dose. Examinated compound did not change the patter of choices in Iowa gambling task. Risky/safe choices in this paradigm is complex behaviour and more studies are needed to explore this aspects of decision-making processes.

P15.15
RELAXIN-3 AGONIST ALTERS SOCIAL CO-SPECIFIC SOCIAL MEMORY AND INCREASES ERK ACTIVATION IN THE AMYGDALA SPECIFIC NUCLEI

Albert-Gascó H, Sánchez-Sarasua S, García-Díaz C, Sánchez-Perez AM and Olucha-Bordonau F

Grupo de Neurobiotecnología, U.P. Medicina, Universitat Jaume I, Castellón, Spain

In mammals, the amygdala is the central core for processing of social and emotional information. Amygdala function is involved in valence processing (positive and negative affective), reward, decision-making and recognition of emotional facial expressions in humans. The ability to identify and recall familiar conspecifics is crucial for a correct social interaction and its absence leads to impaired social abilities. Social memory formation and recall depends on circuits involving amygdala, septum and hippocampus. The nucleus incertus of the pontine tegmentum projects to both amygdala and hippocampus. Neurons of the nucleus incertus produce the neuropeptide relaxin-3, which is co-released with GABA. Thus, relaxin-3 could be one of the modulators of social processing or memory formation. To assess whether the relaxin-3/Relaxin family peptide 3 receptor (RXFP3) system may be affecting social interaction and/or memory we have intracerebroventricularly (icv) infused a RXFP3 agonist and quantified Erk phosphorylation (pErk) by immunoblot and immunohistochemistry in the amygdala. Then, we studied the pErk pattern distribution in the amygdala related to the receptor distribution-density. Finally, we have studied if icv agonist infusion interferes with discrimination between a conspecific subject and an inanimate object or the formation of social memories in a 3-rooms maze paradigm. The results show an increase in Erk activation in amygdala in immunoblot assays 20 minutes after the infusions of agonist when compared to vehicle. Furthermore, the medioventral part of the stria terminalis, the oval nucleus of the stria terminalis, the central amygdala and the posteroventral part of the medial amygdala showed a significant increase or Erk activation. This increase is consistent with the behavioral 3-rooms maze paradigm results where agonist subjects interact more with conspecifics than with inanimate objects but have a clear impairment of social memory. This effect is specific on the social recognition as in the first encounter, in which the animals are challenged to discriminate between subject and object, both vehicle and agonist infused animals showed the same tendency to explore more the co-specific than the inanimated object. These results stand for a first time a role of the NI/RLN3/RXFP3 system in modulating social behavior through specific neuronal types in specific nuclei of the amygdala. As a whole, the system may act as a potential centre of social memory modulator in relation with anxiety and/or stress.

P15.16
AMPLIFICATION OF MGLU5-ENDOCANNABINOID SIGNALING RESCUES BEHAVIORAL AND SYNAPTIC DEFICITS INDUCED BY A DIETARY POLYUNSATURATED FATTY ACIDS IMBALANCE

Manduca A[1,2,3], Bara A[1,2,3], Larrieu T[4,5], Lassalle O[1,2,3], Joffre C[4,5], Layé S[4,5] and Manzoni O[1,2,3]

[1] *INSERM U901, Marseille 13009, France*
[2] *INMED, Marseille, France*
[3] *Université de Aix-Marseille, UMR S901, France*
[4] *INRA, Nutrition et Neurobiologie Intégrée, UMR 1286, 33076 Bordeaux, France*
[5] *Université de Bordeaux, France*

In the last century, the rapid expansion of Western Countries has been associated with drastic changes in the diet reflected by low levels of essential omega-3 polyunsaturated fatty acids (n-3 PUFAs). Our previous studies demonstrated that lifelong n-3 PUFAs dietary deficiency ablates endocannabinoid synaptic plasticity in the medial prefrontal cortex (mPFC) and nucleus accumbens (NAc). These synaptic alterations were correlated with impairments in emotional-related behaviors. However, the onset of these deficits has never been studied.

To address this issue, C57BL6/J mice were fed with an n-3 deficient diet (rich in linolenic acid, LA, 18: 2n-6) starting at postnatal day (PND) 28 until adulthood (PND 90) when they were tested for synaptic plasticity and emotional and cognitive outcomes. Our results showed that starting nutritional deficits in dietary n-3 PUFAs during adolescence decreased n-3 PUFAs levels in both mPFC and NAc, increased anxiety-like behavior and decreased cognitive function in adulthood. Importantly, we discovered that endocannabinoid/mGlu5-mediated long-term depression in the mPFC and NAc was abolished in adult n-3-deficient mice. Additionally, mPFC NMDAR-dependent long-term potentiation was also lacking in the n-3-deficient group. Pharmacological enhancement of the mGlu5/eCB signaling complex, by positive allosteric modulation of mGlu5 or inhibition of endocannabinoid 2-arachidonylglycerol (2-AG) degradation, fully restored synaptic plasticity and normalized emotional and cognitive behaviors in malnourished adult mice.

Our data support a model where nutrition is a key

environmental factor influencing the working synaptic range into adulthood, long after the end of the perinatal period. These findings have important implications for the identification of nutritional risk factors for disease and design of new treatments for the behavioral deficits associated with nutritional n-3 PUFAs' deficiency.

P15.17
WHY DO ONLY SOME ARM RECOVER AFTER STROKE? OF MIRROR NEURONS IN BA44

Anderlini D[1,2] **and Wallis G**[1]
[1] *Centre for Sensorimotor Performance, HMNS, University of Queensland, Brisbane, Australia*
[2] *Neurology Department, Royal Brisbane and Women's Hospital, Brisbane, Australia*

Cortex and cortical function can be regarded as modular, with separate areas involved in processing sensory information and the initiation of motor movement. The blood supply supporting cortex is, like the brain itself, modular. Disruption in blood flow leads to a multiplicity of dysfunctions like the frequent co-occurrence of aphasia and right upper limb hemiparesis.

A review of the stroke literature reveals a correlation between Broca's aphasia and upper limb motor recovery. The precise reason for the correlation is not known but this paper proposes one. Our argument is that speech impairment is indicative of damage to Brodmann area BA45 but that the motor deficits are due to damage to the proximal, but functionally discrete area BA44. BA44 is a multisensory area. But experiments on tone-deaf or stutters, radiological tools like fMRI and DWI, studies of the neuro-ontogeny and development in babies, findings of genetic, epigenetic and embryology, all point to BA44 playing a central role in visuo-motor integration.

We have analysed data from 3780 stroke patients. Two groups were compared: patients with aphasia and right upper limb motor deficit and patients with just motor deficit without aphasia. Patients with Broca's aphasia show slower and poorer motor recovery of right arm compared to non-aphasic ones. Intact BA44 offers a source of unimpaired input to a damaged motor system from primary visual pathways and the cortico basal-ganglia thalamic loop. However, when damaged, the motor system is starved of one important source of signal for effective retraining and recovery.

P15.18
MALTESE STUDY OF INTRACRANIAL VASCULAR MALFORMATIONS

Dalli T, Chircop C and Mallia M
Department of Neuroscience, Neurology Division, Mater Dei Hospital, Msida, Malta

Intracranial vascular malformations (IVMs) are responsible for over a third of spontaneous intraparenchymal brain haemorrhage in the young population. These may cause recurrent intracranial bleeds, focal neurological deficits, seizures and chronic disability. The aim was to study the incidence of arterio-venous malformations (AVMs) and cerebral cavernous malformations (CCMs) in the Maltese population, assess mode of presentation, patterns of interventions, outcomes and follow-up of the lesions. A word search through the radiology information system was carried out, identifying cases of IVMs between January 2008 and October 2016, which presented at Mater Dei Hospital. Brain or dural AVM, carotid-cavernous fistulae and CCM were included in the study. A participant was identified as the 'incident' case at the time of first diagnostic image, be it computed tomography, magnetic resonance (MR) or catheter angiogram. Interventions, follow-ups and complications were noted. Of the 82 participants, 47 had AVMs and 35 had CCMs. The majority of patients with AVM presented with headaches. MRI was the prevalent imaging modality used at the time of diagnosis followed by angiography which was performed in 51% of patients. 61.7% had follow-up imaging within a year since diagnosis. AVM size was documented in 46.8% of cases. 42.6% of patients received radiosurgery with the commonest modality used being gamma knife. The most common complication was haemorrhage. Out of the 35 individuals with a CCM, seizures and focal signs were common presenting symptoms. In 80% of cases, dimensions of the CCM were reported and up to 65.7% of patients were followed-up with further imaging modalities within one year of diagnosis. The majority of patients were followed-up and not offered any interventions.

IVMs may cause significant morbidity in patients and timely recognition is essential. The risk of haemorrhage in patients with AVMs is 2–4% per annum and it is this risk that directs management. Cerebral angiography provides further information to help assess the risk of bleeding. Presently, decisions regarding CCMs are made on a case-by-case basis. There is need for guidelines, which would help direct clinicians on the evidence-based management of AVMs and provide further information on when repeat imaging should be undertaken.

P15.19
MYCOPLASMA PNEUMONIA ASSOCIATED ENCEPHALOPATHY: A CASE REPORT

Dalli T, Chircop C, Galea R and Vella N
Department of Neuroscience, Neurology Division, Mater Dei Hospital, Msida, Malta

Mycoplasma Pneumonia is a common respiratory pathogen causing community-acquired pneumonia. The commonest neurological manifestations of this infection

include encephalitis, myelitis and aseptic meningitis. Encephalopathy is a recognised complication in the paediatric age group. We describe a case of generalised encephalopathy in an adult. 68 years old previously healthy woman presented to accident and emergency after being found unresponsive. She had travelled to Malaysia 4 weeks prior to admission and had a one week history of flu-like symptoms. Clinical examination revealed no localizing signs. She was apyrexial with no neck stiffness, reactive miotic pupils and she withdrew to pain. Intubation and ventilation were required on admission to intensive care where she developed a fever. CT Brain showed evidence of chronic sinusitis but was otherwise normal as was magnetic resonance imaging. She was started on Ceftriazone and Aciclovir empirically. Full toxicology screen, initial inflammatory markers, cerebrospinal fluid and autoimmune screen were negative. CT thorax demonstrated left sided consolidation with positive *M. pneumonia* IgM. Electroencephalogram showed diffuse slowing with sharp triphasic waves in keeping with encephalopathy. She was given sodium valproate empirically. Ceftriazone was changed to Tazobactam and she improved drastically. She became responsive and within one week had no residual neurological deficit.

M. pneumonia was undetected in the CSF, pointing against direct invasion of the CNS. Immune mediated processes occurring after infection could be a likely explanation although the patient improved without the use of steroids. Further research is required in determining whether encephalopathy in such rare presentations is secondary to an immune-mediated process.

P15.20
BRAIN TISSUE LEVEL OF NERVE GROWTH FACTOR IN A MOUSE MODEL OF CUPRIZONE-INDUCED DEMYELINATION

Yarim GF[1], Karayigit MO[2] and Yarim M[3]
[1]*Department of Biochemistry, Faculty of Veterinary Medicine, Ondokuz Mayis University, Samsun Turkey*
[2]*Department of Pathology, Faculty of Veterinary Medicine, Cumhuriyet University, Sivas, Turkey*
[3]*Department of Pathology, Faculty of Veterinary Medicine, Ondokuz Mayis University, Samsun, Turkey*

Demyelination refers to the destruction of myelin sheath and is caused by inflammatory events, infectious and autoimmune diseases and toxic agents that damage the myelin sheath. Nerve growth factor (NGF) is a neurotrophic factor that is important for the development, maintenance and survival of the neurons. The aim of this study was to determine the brain level of NGF in a mouse model of cuprizone-induced demyelination. Twenty male C57BL6 mice, 6–8 weeks old were used in this study. To induce demyelination, 10 mice were fed chow containing 0.3% cuprizone and control group were fed standard chow during 5 weeks. Mice were sacrificed under xylazine and ketamine anaesthesia and brain tissue removed at necropsy. Brain tissues were used for histopathological examinations and NGF analyses. For determination of demyelination, corpus callosum sections stained with Luxol fast blue. Protein extraction was performed from brain tissues for NGF analysis. In brain tissue extracts, NGF concentrations were measured by enzyme-linked immunosorbent assay. Brain tissue NGF concentrations in demyelinated group and control group were 0.81 ± 0.13 ng/g brain tissue and 2.23 ± 0.45 ng/g brain tissue, respectively ($p < 0.05$). In conclusion, findings from the present study suggest that demyelination is associated with decreased NGF level in brain tissue. This research was supported by Ondokuz Mayis University Research Fund (PYO.VET.1904.09.001).

P15.21
NEUROPROTECTİVE EFFECTS OF EPİDERMAL GROWTH FACTOR

Yarim GF[1], Yarim M[2], Filiz K[1] and Torunoglu E[1]
[1]*Department of Biochemistry, Faculty of Veterinary Medicine, Ondokuz Mayis University, Samsun Turkey*
[2]*Department of Pathology, Faculty of Veterinary Medicine, Ondokuz Mayis University, Samsun, Turkey*

Epidermal growth factor (EGF) is a growth factor in protein structure that stimulating division, differentiation, survival, proliferation, growth and migration of the cells which is involved in many physiological and pathological processes of the organism. EGF exerts its biological effects through EGF receptor (EGFR) which a transmembrane protein. Epidermal growth factor receptors are located on the cell surface of many tissues that include lung, stomach, duodenum, pancreas, kidney, pituitary gland, thyroid gland, mammary gland, ovary, uterus, placenta, cornea and glia. EGF activates the mitogen-activated protein kinase, extracellular signal-regulated kinase 1/2 and phosphatidylinositol 3 kinase (PI3K)/Akt signaling pathways stimulating cell proliferation and survival through binding to EGFR. EGFR plays a role in proliferation and differentiation of astrocytes and survival of postmitotic neurons. EGFR is also known to have an important role in oligodendrocyte development. In acute spinal cord injury, EGF treatment alleviates the deterioration in the blood-spinal cord barrier permeability via PI3K/Akt/Rac1 pathway and increases locomotor activity. Intranasal heparin-bound EGF treatment increases the formation of new oligodendrocytes from progenitor cells and induces functional recovery in newborn brain injury model. Plasma EGF levels is suggested that a biological marker of cognitive decline in patients with Parkinson disease and

Alzheimer disease. EGF is evaluated as a key molecule for remyelination in patient with multiple sclerosis. EGF treatment is seen as a novel approach to the treatment of nervous system diseases.

P15.22
INVESTIGATION OF BRAIN-DERIVED NEUROTROPHIC FACTOR CONCENTRATION OF BRAIN TISSUE IN NEUROINFLAMMATION MODEL

Filiz K[1], Yarim GF[1], Yarim M[2] and Karaca E[2]
[1] *Department of Biochemistry, Faculty of Veterinary Medicine, Ondokuz Mayis University, Samsun, Turkey*
[2] *Department of Pathology, Faculty of Veterinary Medicine, Ondokuz Mayis University, Samsun, Turkey*

Neuroinflammation is the inflammation of nervous tissue that can leads to neurodegeneration. Brain-derived neurotrophic factor (BDNF) is a neurotrophin which affectes growth, function and survival of neurons in peripheral and central nervous system, enhances the stabilization of synapses, regulates synaptic function and branching of dendrites and axons. BDNF is believed to be involved in the pathophysiology of central nervous system diseases associated with neuroinflamation. Possible changes in decline of BDNF in brain tissue with neuroinflammation has not been reported before. The aim of the present study was to determine the concentration of BDNF in brain tissue of mice with neuroinflammation.

A total of 20, 8–10 weeks-old, male Swiss albino mice were used in this study. Twenty mice were randomly divided into two groups which received either intraperitoneal injection single dose of 0.9% NaCl solution (control group) or 5 mg/kg lipopolysaccharide (neuroinflammation group). Mice were sacrificed by ketamine and xylazine anesthesia after 48 hours. Lesions that related with neuroinflammation in the brain tissue were determined with histopathological examination. The brain tissue BDNF concentrations were determined using mouse specific enzyme-linked immunosorbent assay kit following the procedure as described by the manufacturer. The brain tissue concentrations were determined to be 0.33 ± 0.08 ng/mg protein and 0.65 ± 0.08 ng/mg protein in the neuroinflammation group and control group, respectively ($p < 0.05$). These findings suggest that BDNF concentration may be used as a marker of disease characterized by neuroinflammation in the central nervous system. This research was supported by Ondokuz Mayis University Research Fund (PYO.VET.1904.15.012).

P15.23
ACTIVATION OF IMMEDIATE EARLY GENES IN THE TELENCEPHALON OF RATS FOLLOWING LESIONS OF THE NUCLEUS INCERTUS AFTER SOCIAL RECOGNITION TEST

Castro-Salazar E[1,2], García-Díaz C[1], García-Avilés A[1], Sánchez-Catalán MJ[1], Sánchez-Sarasúa S[1], Albert-Gascó H[1], Sánchez AM[1], Ros-Bernal F[1] and Olucha-Bordonau FE[1]
[1] *Universitat Jaume I, Castellón de la Plana, Spain*
[2] *Universitat de Valencia, Valencia, Spain*

The specific NI projections to the amygdala may indicate a putative role of this tegmental nucleus in social behaviour. To study a specific participation of this system in modulating social recognition, the patterns of c-fos activation were measured in different telencephalic areas following NI lesions after a 3-rooms maze. Lesions were performed in 300–350 g male wistar rats by stereotaxic injection of quinolenic acid in the NI. Sham animals received the same injection in the cerebellum. Animals were allowed to recover for 7 days. The 3-rooms maze consisted in two trials. In a first trial, the subject had free access to two rooms in one room, there was a cospecific and in the other room an object. In the second trial, the object was substituted by a novel conspecific. Sham cases tended to visit more the novel subject than the familiar one, but lesioned animals did not differentiate between the familiar and the novel. animals were anesthetized intracardially perfused with saline and fixative 1 h after the end of the behavioural paradigm. Then, ICC for c-fos was performed in 40 µm sections. Images were taken at 20x magnification for selected areas of the hippocampus, amygdala and septum and quantified by using ImageJ software.

When applied the t-test, significative differences were observed in the horizontal limb of the diagonal band and in the nucleus triangularis septalis. There were a significant difference between sham and lesion group on piriform cortex and in the central nucleus of the amygdala. The rest of amygdala nuclei did not show significant differences. We also observed significant differences between both lesioned and sham groups in CA1, CA2, and CA3 of hippocampus. However, the granule layer of the dentate gyrus did not displayed significant differences. These results point at this structures as targets modulated by the NI during performance of social recognition test.

P15.24
WIN55,212-2 ACUTE ADMINISTRATION INDUCES BEHAVIORAL AND SYNAPTIC CHANGES IN A SEX- AND AGE-DEPENDENT MANNER

Borsoi M[1], Manduca A[1], Bara A[1], Lassalle O[1], Pelissier-Alicot AL[1,2] and Manzoni O[1]
[1] *Inserm Unit 901, Inmed, Marseille, France, Aix-*

Marseille University, Marseille, France
[2]*APHM, CHU Timone Adultes, Service de Médecine Légale Marseille, France*

Marijuana use in adolescents has been associated with impairments in cognition related with several domains influenced by the prefrontal cortex (PFC) such as social behavior, an important form of social interaction in mammals. Previous work has suggested that the endocannabinoid system is involved in its modulation and that it seems to be sex-dependent. We combined electrophysiological and behavioral approaches to determine the effect of the synthetic cannabimimetic WIN55,212-2 on PFC synaptic plasticity and social interaction. Male and female rats at different ages received a single injection of WIN55,212-2 (s.c. 2 mg/kg) 24 h before behavioral evaluation and slice electrophysiology.

The results show that WIN55,212-2 administration altered N-methyl-D-aspartate receptor NMDA receptor (NMDAR)-dependent long-term potentiation (LTP) and social interaction. Both LTP magnitude and social behavior in male were affected while female juvenile rats were resistant to the cannabis-like compound. In marked contrast pubescent rats of both sex were not affected. Finally, adult rats also presented sex-dependent alterations in LTP.

These results suggest that the impairment of PFC plasticity induced by a single exposure to a cannabimimetic co-variates with social behavior changes. These alterations differentially affected male and females rats, indicating a sex-dependent modulation of PFC plasticity and social behavior by cannabis.

P15.25
ROLE OF THE NUCLEUS INCERTUS IN THE MODULATION OF THE SOCIAL RECOGNITION IN ADULT MALE RATS

García-Díaz C[1], Castro-Salazar E[1,2], García-Avilés A[1], Sánchez-Catalán MJ[1], Sánchez-Sarasúa S[1], Albert-Gascó H[1], Sánchez AM[1], Ros-Bernal F[1] and Olucha-Bordonau FE[1]
[1] *Universitat Jaume I, Castellón de la Plana (Spain)*
[2] *Universitat de Valencia, Valencia (Spain)*

Modulation of hippocampal and amygdala function depends on ascending subcortical projections. We have focused our research on the nucleus incertus (NI), a tegmental centre involved in telencephalic modulation. NI displays projections to the hippocampus, amygdala, prefrontal cortex and medial septum. NI uses GABA as neurotransmitter that colocalizes with the neuropeptide relaxin-3 (RLN3). RLN3 belongs to the superfamily of relaxin and insulin peptides. Most neurons expressing RLN3 in the brain are concentrated in the NI. Tracing studies suggest that NI pathways may play a relevant role in the integration of information related to memory and attention. In addition, the occurrence of RLN3 fibers in the medial amygdala may indicate a relevant role of this system in modulating social behaviour. Thus, the aim of this work is to study the putative effect of NI inactivation on social recognition by using the 3 rooms maze. In this study, adult male wistar rats were NI lesioned by stereotaxic infusion of quinolenic acid. After 1 week of recovery from surgery. the social recognition of three chambers was done. The paradigm was developed in two trials. On the first trial, the problem subject was allowed to explore between two chambers, in one chamber, there was an inanimated object and in the other one another subject. On the second trial, the object was substituted by a new subject so that the problem subject was allowed to explore the familiar and the novel subjects.

Both, lesioned and sham animals were able to readily differentiate between the subject and the object on the first trial. However, sham animals spent significantly more time exploring the novel subject compared to the familiar one during the second trial. In contrast, the lesioned animals were unable to differentiate between familiar and novel subjects and explore equal time both subjects in the second trial. These results clearly demonstrate a role of the nucleus incertus in social recognition in rats.

P15.26
IS BIOLOGICAL MOTION VIEWPOINT DEPENDENT?

Ballarini N and Thornton IM
Department of Cognitive Science, University of Malta, Msida, Malta

There has been much debate as to whether and how objects can be recognized across viewpoint changes. Here we ask whether viewpoint changes affect performance when participants make judgements about human actions depicted as point-light stimuli. Previous research has suggested that bodies may be "special" objects and may thus be immune to such viewpoint costs.

We used a concurrent matching task in which 3 dynamic point-light figures performed familiar actions taken from a standard biological motion database. On each trial the action performed by the central "target" figure was also performed by one of the two flanking figures. The participants task was to make a speeded left/right response to indicate which flanker was copying the target. The depth orientation of the target figure was randomly assigned and the matching flanker could either have the same orientation or appear with an offset of 45° or 90° relative to the target. The orientation of the non-matching flanker was random as were the step cycles of all three figures.

We found viewpoint changes affected both speed and accuracy. Repeated measures ANOVAs indicated significant main effects of viewpoint for both dependent measures. Post-hoc analysis showed that participants were faster and made fewer errors when the target and matching flanker had the same depth orientation compared to either viewpoint change condition. There were clear trends for better performance in the 45° compared 90° conditions, although these did not reach significance.

In the current experiment we have shown clear costs in recognizing the same action across viewpoint changes. This indicates that the recognition of human bodies depicted as biological motion stimuli is viewpoint-dependent, as with many other types of object. We also suggest that concurrent matching is a flexible tool for exploring biological motion as decisions can be made on a variety of actions without the need for explicit action-naming or training.

P15.27
APAMIN-SENSITIVE SK CHANNELS MODULATE TONIC AND PHASIC DOPAMINE RELEASE IN THE NUCLEUS ACCUMBENS SHELL: A MICRODIALYSIS STUDY

Miliano C[1], Valentini V[1], Piras G[1] and Di Chiara G[1,2]

[1] *Department of Biomedical Sciences, University of Cagliari, Italy*

[2] *CNR Institute of Neuroscience, Cagliari Section, University of Cagliari, Italy*

Midbrain dopaminergic neurons have two different firing patterns: single spike firing, associated with tonic dopamine (DA) release and burst firing, associated with phasic DA release. Burst firing in DA neurons is controlled by apamin-sensitive Ca++-activated K+(SK) channels. Electrophysiological studies showed that the blockade of SK channels by apamin increases DA burst firing while activation reduces it. In order to demonstrate that microdialysis technique is able to detect phasic dopamine release, we evaluated the effect of intra-ventral tegmental area (VTA) and intra-substantia nigra (SN) administration of apamin on dialysate DA in the striatum.

By *in vivo* microdialysis studies, we locally injected apamin (1.7 pmol/1 µl; 3.3 pmol/1 µl) or vehicle into the VTA and the SN, and we measured extracellular DA levels in the ventral striatum (nucleus accumbens, shell and core) and in dorsal striatum (dorsomedial and dorsolateral CPu). Intra-VTA apamin, at doses of 1.7 and 3.3 pmol, dose-dependently increased dialysate DA in the NAc shell but not in the NAc core. Doses of 3.3 pmol apamin increased NAc shell dialysate DA by 75% over basal after 40 min, reaching a plateau of 100–125% over basal at 80 minutes post-drug. The infusion of the dose of 1.65 pmol did not affect the dopamine transmission on dorsal CPu. Intra-SN apamin at the highest dose (3.3pmol) increased extracellular DA levels in dorsomedial CPu but not in the dorsolateral CPu, nor in the ventral striatum.

Collectively these observations are consistent with the idea that NAc shell dialysate DA can provide a correlate of phasic stimulation of in vivo DA transmission as a result of activation of DA neuron burst firing. Our observations contradict the common belief that brain microdialysis only reflects the tonic modality of DA transmission *in vivo*.

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